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ERRATA

- ✓ Page 16, line 8, for "Trinidad" read "Trinidad"
 - ✓ Page 41, line 13 (sixth reference), for "NOTLEY, F. B. (1941). *Antestia* in the Northern Province of Tanganyika.—E." read "NOTLEY, F. B. (1933). The control of *Antestia* in wetter districts.—*Bull. Dep.*"
 - ✓ Page 167, 11 lines from end, for "*G. p. fuscipes*" read "*G. palpalis fuscipes*"
 - ✓ Page 170, Table II (heading), for "second series" read "second replicate"
 - ✓ Page 343, 13 lines from end, after "*A. natronius*" insert "Edw."
 - ✓ Page 346, line 37, for "genus" read "subgenus"
 - ✓ Page 416, first line, after "*G. palpalis*" insert "(R.-D.)"
 - ✓ Page 479, paragraph 7, line 2, page 486, paragraph 4, line 2, and page 487, paragraph 4, first line, for "thelytokous" read "thelytokous"
 - ✓ Page 483, lines 46 and 47, for "*S. lepidus*" read "*S. flavescens*"
 - ✓ Page 621, first footnote, for "University of Sydney, N.S.W." read "University of New South Wales, Sydney"
- Pages 635-645, for "*Piezotrachelus varium*" read "*Piezotrachelus varius*"

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FURTHER CONSIDERATIONS REGARDING THE REPELLENCY
OF SPRAY COMPONENTS.

By B. HOCKING

Department of Entomology, University of Alberta.

Several components of insecticidal sprays have been shown to have an undesirable repellent effect on the insects that they are intended to kill (Hocking & Lindsay, 1958). This effect is here explored further and ways of circumventing this difficulty are considered. An attempt has been made to narrow down the ingredients responsible for the effect and consideration given to the possibility of finding substitutes for them with less repellent or even attractive properties. The possibility of using specific attractive additives is also considered.

Materials and methods.

The diesel fuel oil and the methylated naphthalene auxiliary solvent Velsicol AR-50 used in the work already referred to, and a similar additional auxiliary solvent, Velsicol AR-55, with a somewhat higher initial boiling point, were studied. Each of these materials was broken down by fractional distillation into ten fractions. Groups of these fractions were then recombined for further testing.

The following six materials in which DDT is highly soluble (Gunther, 1945; Jones, Fluno & Hendrick, 1945; Buxton, 1945) were also investigated: cyclohexanone, ethylene chloride, tetrahydronaphthalene, benzyl benzoate, acetone, and dimethyl phthalate. The last of these materials was included in order to see if its well-known properties as a mosquito repellent could be demonstrated by this type of test. An additional material widely used as a fly repellent, a butoxy-polypropylene glycol, was also included for the same reason; although it is claimed to be a good solvent, data on the solubility of DDT in it are not available. Methyl ethyl ketone, 1,4-dioxane, and ethyl acetate were also tested but proved too toxic to give results.

The T-tube apparatus (Hocking & Lindsay, 1958) was used, with the following modifications. Laboratory air supply was used; the air stream was divided after the humidifying tower by a Y-tube; the two streams were independently

controlled by screw clamps. A tapered-tube flow meter was inserted in each stream, one of which then passed through the tower of odorant and the streams were then reunited. With materials which were sufficiently volatile to give air saturated with the vapour from a single 250 ml. tower at the maximum rate

TABLE I.

Olfactory responses of adults of some Diptera to the vapour of spray components and other materials.

SPRAY COMPONENTS (tested with *Drosophila melanogaster*):

	Light fraction	Middle fraction	Heavy fraction
Velsicol AR-50			
Boiling point	390-450°F.	465-515°F.	Above 530°F.
Olfactory response	-0.73 ± 0.029 (4)	-0.66 ± 0.065 (4)	-0.44 ± 0.036 (5)
Velsicol AR-55			
Boiling point	415-460°F.	470-520°F.	Above 530°F.
Olfactory response	-0.76 ± 0.067 (4)	-0.63 ± 0.038 (4)	-0.35 ± 0.034 (4)
Fuel oil			
Boiling point	352-406°F.	415-452°F.	Above 461°F.
Olfactory response	-0.75 ± 0.017 (4)	-0.69 ± 0.051 (4)	-0.57 ± 0.019 (4)

OTHER MATERIALS:

	<i>D. melanogaster</i>	<i>Musca domestica</i>	<i>Aedes aegypti</i>
Acetone			
Saturated vapour	-0.62 ± 0.058 (6)	—	—
50% saturation	-0.39 ± 0.041 (4)	—	—
Cyclohexane	-0.56 ± 0.033 (4)	—	—
Ethylene chloride	-0.62 ± 0.014 (4)	—	—
Tetrahydronaphthalene			
Saturated vapour	-0.82 ± 0.008 (4)	—	—
50% saturation	-0.62 ± 0.051 (4)	—	—
Odour of <i>D. melanogaster</i>	$+0.33 - 0.11$ (5)	—	—
Benzyl benzoate	—	-0.16 ± 0.06 (4)	—
Dimethyl phthalate	—	-0.67 ± 0.031 (4)	-0.76 ± 0.022 (4)
Butoxy-polypropylene glycol	—	$+0.39 \pm 0.45$ (11)	$+0.30 \pm 0.023$ (3)

Each entry represents the mean \pm standard error of the mean, followed (in brackets) by the number of readings.

(0 = neutral, +1.0 = maximum attractiveness, -1.0 = maximum repellency.)

of air flow used, it was then possible to control the vapour concentration simply by controlling the proportion of the air passing over the odorant. Two materials were tested at concentrations below saturation. All attempts to saturate air with materials of low volatility such as dimethyl phthalate failed at the rates of flow which were necessary for this work. With these materials the vapour concentration obtained is thus unknown, although it could be duplicated approximately.

All the tests described here were done with *Musca domestica* L. of the Suffield strain, *Drosophila melanogaster* Mg. or *Aedes aegypti* (L.), all laboratory reared.

During the course of the work, observations on the behaviour of *Drosophila*, and in particular on the behaviour in the T-tube, suggested that this was affected by the odour of the insect itself. This is quite strong to the human nose, very distinctive, and undoubtedly familiar to most workers with the insect. The possibility that this might serve as a specific attractant prompted some tests with this odour. Odorous air was obtained by confining a large population of flies in a five-gallon bottle for two to three days. The air in the bottle was then used for a test by running water into the bottle to drive the odorous air out through the T-tube.

Results.

The results of all tests are summarised in Table I. The method of calculation and expression is the same as that used previously (Hocking & Lindsay, 1958).

In nearly every series of tests with fractions of spray components, fewer flies reacted downwind in the later tests, that is, the material became less repellent. This was probably due, as in the earlier work, to the selective evaporation of the more volatile components in the early tests.

In all tests an initial count was made a few minutes after the flies had been put into the apparatus. During this time usually about 70 to 80 of the 100 flies used would respond spontaneously and get into either the upwind or the downwind chambers. After this count was made, the apparatus was agitated for a minute or two to stimulate reluctant specimens into a response. The total responding was then usually 95 to 100 per cent. Results after agitation nearly always gave a smaller proportion of the insects moving upwind, presumably because at least some of the less energetic specimens stimulated into response in this way were carried downwind by the air stream. In a few tests, however, nearly all the specimens reacting on agitation moved upwind, even in one test in which the general reaction was downwind. I have no explanation for this. Figures in the table are olfactory response figures based on the spontaneous reactions.

Discussion.

It is noteworthy that for all of the spray components the repellency is inversely related to the boiling point, and that even the fractions with the highest boiling points are decidedly repellent. Of the two methylated naphthalene solvents, however, that with the higher initial boiling point gave the more repellent light fraction, but the difference is not significant. It seems that the repellent effects of both the fuel oil and the methylated naphthalenes are non-specific, both between insects (see Hocking & Lindsay, 1958) and between related chemical compounds, and the degree of repellency depends simply on the volatility—and hence the concentration. This is borne out by the decrease in repellency in the later tests with the same material and by the reduced repellency of both acetone and tetrahydronaphthalene at 50 per cent. saturation. But the tests with materials of low volatility, benzyl benzoate, dimethyl phthalate, and butoxy-polypropylene glycol show equally clearly that it is not a simple broad relationship between volatility and repellency. Rather, as Dethier has suggested (1947), that for each substance or class of substances, there is a vapour concentration of

maximum olfactory attractiveness. At concentrations above this level, attractiveness decreases and becomes repellency which continues to increase with concentration. The concentration which has maximum attractiveness may, however, be too low for detection or measurement.

It is interesting to note that the butoxy-polypropylene glycol, which is advertised as a repellent ingredient for livestock sprays, exhibits consistent olfactory attractiveness to both *Musca* and *Aedes aegypti*. The advantages conferred by the incorporation of this material in livestock sprays (Bruce & Decker, 1955; Granett, Haynes & Helm, 1951) may be attributable to differences in the behaviour of other species, to effects in combination with other ingredients, to stimulation of the L or rejection fibre of the gustatory receptors (Hodgson & Roeder, 1956), or perhaps to effects on the general chemical sense, as has been suggested as a contributory factor for the action of mosquito repellents (Kalmus & Hocking, 1960).

Carrier oils and solvents are required in such quantities in insecticidal sprays that there seems little hope of an economic non-repellent material, especially since rather high chemical purity is likely to be called for. Two possibilities remain.

Firstly, to counteract the repellent effect by the use of a specific attractive additive. The results with *Drosophila* odour suggest that this might be possible for some species. If possible, it would probably have the added attraction of specificity. It should be noted that in the tests with *Drosophila* there was no significant difference between the sex ratios of the groups going upwind and downwind. This then is a species attractant, not a sex attractant, but synthetic sex attractants might also be used in this capacity as the chemistry of them comes to be better understood (Hecker, 1958). Similar species attractants have also been reported in grasshoppers (Norris, 1954) and cockroaches (Hocking, 1958) and seem likely to be widespread in insects.

Secondly, this repellent property of spray ingredients could, as Ginsburg (1935) has suggested, prove an advantage. There are many circumstances, inimical to the use of toxicants, where a space repellent spray, formulated from the lighter fractions of the solvent materials, might be valuable.

Summary.

The apparatus previously used to demonstrate the repellent properties of DDT, fuel oil and methylated naphthalene auxiliary solvents towards certain Diptera was used in further studies of olfactory repellency in an attempt to narrow down the ingredients responsible for this effect, and consideration was given to the possibility of finding substitutes for them with less repellent or even attractive properties.

Light, medium and heavy fractions were obtained by fractional distillation of fuel oil and each of two methylated naphthalene auxiliary solvents. In all these spray components the repellent properties are inversely related to the boiling point, and appear to be non-specific both chemically and by insects. Tests with materials of low volatility, however, showed that the effect is not a simple broad relationship between volatility and repellency.

Butoxy-polypropylene glycol, widely used as a 'repellent' ingredient of livestock sprays, shows olfactory attractiveness to *Musca domestica* L. and *Aedes aegypti* (L.). The odour of *Drosophila melanogaster* Mg. was shown to be attractive to the insect, regardless of sex.

It is concluded that there seems little hope of an economic non-repellent material.

It is suggested that the repellency of spray components might be counteracted by the addition of specific attractants, sex or other, to spray formulae.

Alternatively, a useful space repellent spray, containing no toxicant, might be formulated from the lighter fractions of the solvent materials.

Acknowledgements.

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THE DEVELOPMENT AND OVIPOSITION OF *ORYZAEPHILUS* SPP. ON UNREFINED SUGARS.

By MICHAEL H. BREESE

E.H.V.

*Regional Research Centre, Imperial College of Tropical Agriculture,
Trinidad, W.I.*

The biology of *Oryzaephilus surinamensis* (L.) and *Oryzaephilus mercator* (Fauv.) has been studied in detail by Howe (1956) who listed the products on which these beetles had been imported into Britain over a period of 15 months. Howe showed that *O. surinamensis* is associated mainly with starchy foods and *O. mercator* with oilseeds and their derivatives, but makes no mention of either of these species having been found on any type of sugar, although Wheeler (1921) lists this commodity among those on which *O. surinamensis* occasionally "lives". This is probably because, in Britain, even raw sugar is not normally regarded as being susceptible to infestation and is not regularly inspected. In the West Indies, however, where there is a considerable direct consumption of unrefined sugars, stocks in factory stores and wholesalers' warehouses often carry surface and peripheral infestations of Cucujid beetles. The Cucujid most commonly found is *Nausibius clavicornis* (Kug.), and Breese & Wise (1959) have shown that this beetle is able to complete its life-history on certain types of unrefined sugar. Often occurring with *N. clavicornis* in sugar, and sometimes alone, is *O. mercator*, but infestations of *O. surinamensis*, either alone or with the other species, are much less common. In Trinidad, *N. clavicornis* has not been found on any commodity other than sugar, but these species of *Oryzaephilus* probably infest a greater range of produce than any other pest of stored products. Their presence in sugar is usually taken as an indication of cross-infestation, but whereas this may be true in granulated (refined) sugar, the fact that both larvae and adults of *O. mercator* have been found in unrefined sugars (Breese & Wise, 1959) suggests that this species, at least, may be able to breed in them. As Breese & Wise have pointed out, the actual damage (in terms of loss of sucrose) caused by this infestation is probably small in comparison with that which takes place increasingly with the uptake of water in humid climates, but the possibility of 'carry over' in a commodity not normally considered as being subject to infestation is important. For this reason the oviposition of *O. mercator* and *O. surinamensis* and their ability to complete their life-history and in two types of unrefined sugar were studied.

Materials and methods.

Parent stocks of both species were collected from infested commodities (mostly rice) on the Port-of-Spain wharves and were bred in an incubator at 25°C. and 75 per cent. R.H., on a mixture of 9 parts rolled oats, 2 parts wholewheat flour and 1 part powdered yeast.

The average size of individuals of both species fell outside the ranges quoted in the literature, being rather smaller, especially for *O. surinamensis*. Although Back & Cotton (1926) put the average length of *O. surinamensis* at about one-tenth of an inch (2.54 mm.), Slow (1958) gives the size range for this species as from 2.75 mm. to 3.25 mm., and from about 3 mm. to nearly 4 mm. for *O. mercator*. No significant increases in size were obtained when the beetles were bred through

many generations on several different food materials including Haydak's formula (Haydak, 1936), and the average sizes were, *O. surinamensis*: ♂ 2.20 mm., ♀ 2.26 mm., and *O. mercator*: ♂ 2.87 mm., ♀ 2.77 mm.

Limited observations on the biology of the two species on oat flour (finely ground rolled oats with five per cent. powdered yeast) were undertaken using techniques similar to those employed by Howe (1956). These showed that the total development period of individually reared adults was similar to that recorded by other workers at similar temperatures and humidities (see Table I). Fourteen

TABLE I.

Pre-adult development period of *Oryzaephilus* spp. on different foods at 25°C.

Food	R.H. per cent.	Development period (days)	
		<i>O. surinamensis</i>	<i>O. mercator</i>
Oat flour and yeast ..	75	29-31	30-32
Wheatfeed ¹	75	approx. 33	approx. 34
Rolled oats ²	78.8	30.3	—

¹ Howe (1956) by interpolation.

² Thomas & Shepard (1940).

larvae of each species were studied, and with *O. mercator* 13 of these became adults, but with *O. surinamensis* only 11 larvae reached the pupal stage and only nine became adults. Two adults of *O. surinamensis* died while emerging from the pupal integument. The apparent greater vigour of the stock of *O. mercator* was also reflected in the oviposition cycles. Whereas Howe found that *O. surinamensis* could lay from six to 10 eggs a day on wheatfeed at 30°C. and 70 per cent. R.H., in the present work the rate for this species on wholewheat flour (plus five per cent. yeast) rarely exceeded one egg a day and hardly any improvement was shown on oat flour (fig. 1a). *O. mercator*, on the other hand, maintained on wholewheat flour an oviposition rate similar to that observed by Howe with wheatfeed, but on oat flour a much higher rate exceeding six eggs a day for the greater part of the ten-week observation period (fig. 1b). The over-all viability

TABLE II.

Average total egg-production and over-all viability of females of *Oryzaephilus* spp. on different foods.

Species	Food	Oviposition period (weeks)	Total egg- production per ♀	Viability per cent.
<i>O. surinamensis</i>	Wholewheat flour and yeast	9	40	81
	Oat flour and yeast	9	51.6	83
	Wheatfeed ¹	ca. 9	375	95
	Split maize ²	ca. 20½	285*	—
<i>O. mercator</i>	Wholewheat flour and yeast	6	97	88
	Oat flour and yeast	9	354	88
	Wheatfeed ¹	ca. 12	200	95

¹ Howe (1956) at 30°C. and 70 per cent. R.H.

² Back & Cotton (1926). Conditions not controlled.

* Highest egg total.

of eggs laid by *O. mercator* on both wholewheat flour and oat flour was 88 per cent., whereas that of eggs of *O. surinamensis* was 81 per cent. on wholewheat flour and 83 per cent. on oat flour.

The results obtained from observations on the oviposition cycle are summarised and compared with the findings of other workers in Table II. Particularly

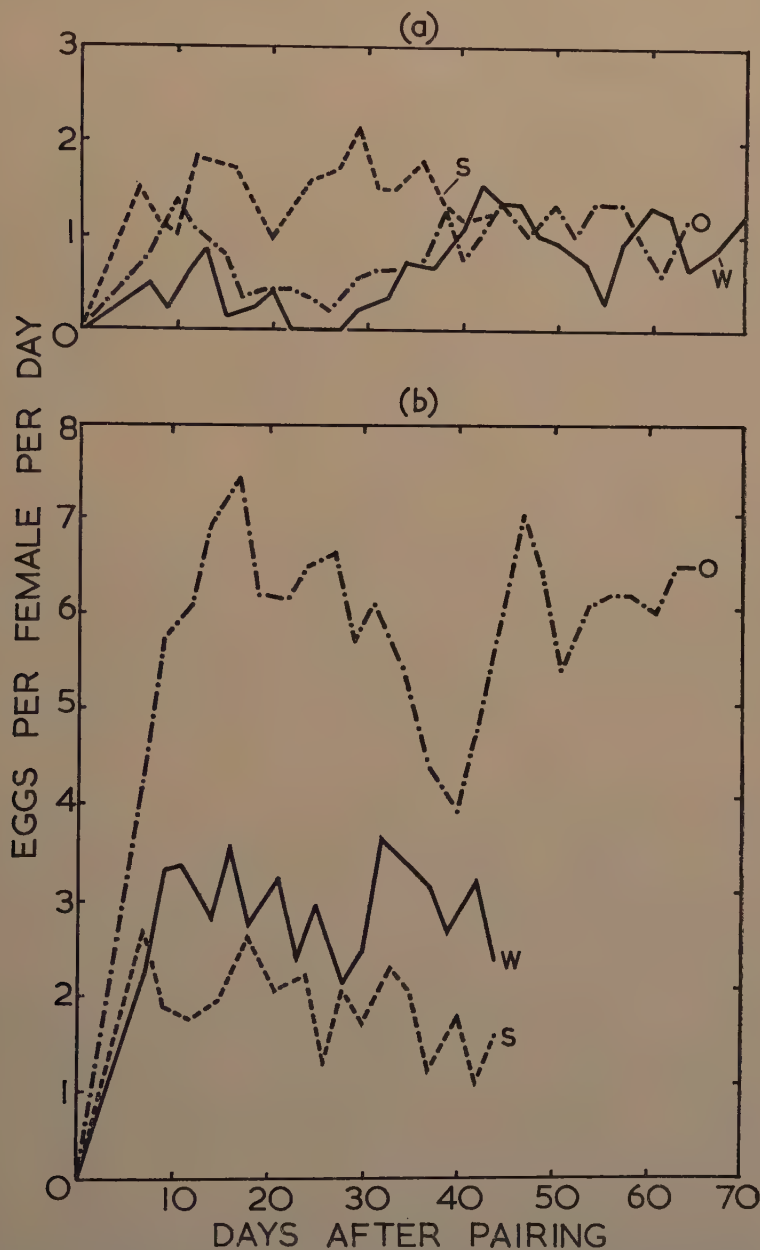


Fig. 1.—Oviposition rates of (a) *Oryzaephilus surinamensis* and (b) *O. mercator*. O, on oat flour; W, on wholewheat flour; S, sugar-reared females on oat flour.

striking is the low egg-production of females of *O. surinamensis*. In case this was due to shortcomings in techniques, the experiments were carefully repeated with freshly bred adults of new stock, using both wholewheat flour and oat flour, but no significant improvements were recorded. The greater vigour of the stock of *O. mercator* demonstrated by these experiments is in keeping with the general impression, gained from infestation surveys, that this species is the dominant one on most stored cereal and farinaceous foodstuffs in Trinidad.

Observations on unrefined sugar.

Fresh samples of raw sugar ('export grey') and good quality yellow-crystal ('Demerara') sugar having sucrose contents of 96 and 97.3 per cent., respectively, were obtained and kept in tightly sealed Kilner jars.

In oviposition studies, eggs were recovered from sugar by the filtration method described by Breese & Wise (1959). All experiments were conducted at 25°C. and 75 per cent. R.H. Full-grown larvae taken from stock cultures were placed individually on a small quantity of food in 5-ml. tubes and the adults allowed to emerge. When sufficient adults from three to seven days old were available they were sexed and paired and the pairs were then kept separately on the stock food for five days. The pairs were then transferred to 3" x 1" tubes containing a level quarter-teaspoon of raw or yellow-crystal sugar. Seven tubes were used with each type of sugar. Eggs were first recovered two days later, *i.e.*, seven days after the beetles had been paired, and thereafter three times weekly. Egg counts were continued for up to 70 days after pairing.

Oviposition and viability of eggs in *O. surinamensis*.

After the eggs that had matured before the pairs were transferred to sugar had been laid, oviposition virtually ceased, and on each type of sugar the total number of eggs laid by all seven beetles between the thirteenth and seventieth day after pairing was only eight. The average number of eggs laid per female in 70 days was, on raw sugar, 4.8 and on yellow-crystal sugar, 3. Whereas the viability of eggs laid in the first two weeks of oviposition was 91 per cent. on raw sugar and 82.6 per cent. on yellow-crystal sugar, only about half of the small number of eggs laid during the remaining period on both types of sugar hatched.

Oviposition and viability of eggs in *O. mercator*.

As with *O. surinamensis*, there was a rapid fall-off in the rate of egg-laying during the first two weeks of oviposition, but on both sugars several females continued to lay sporadically during the remainder of the observation period. On raw sugar, two females maintained a low but almost continuous rate of egg-laying and after 70 days had laid 29 and 31 eggs, respectively. The average number of eggs laid per female was 16.6 on raw sugar and 12.4 on yellow-crystal sugar. As with *O. surinamensis* there was a marked reduction in the viability of eggs laid on yellow-crystal sugar after the first two weeks, the drop being from 86 per cent. to 50 per cent. On raw sugar the fall in viability was not quite so marked, and 66 per cent. of the eggs laid after the first two weeks of oviposition hatched, compared with 92 per cent. of the eggs laid previously.

Development of larvae.

Larvae less than 24 hours old, hatched from eggs laid on sugar at the beginning of the oviposition cycle, were placed individually in 5-ml. vials containing a level quarter-teaspoon of raw or yellow-crystal sugar. The tubes were closed with cotton-wool plugs and 20 were used with each type of sugar. Tubes were

examined every two days, but the plugs were not removed nor the contents disturbed in any way, unless a cast skin was noticed and thereupon carefully extracted. A second level quarter-teaspoon of sugar was added to each tube between 24 and 30 days after the start of the experiment. A moult was recorded for a larva whenever a cast skin was found but because skins are difficult to find, especially in raw sugar, the actual time of the moult could not be definitely ascertained. When an adult emerged or a larva died, the sugar in the tube was dissolved and head capsules were recovered by filtration. In this way an indication of the number of moults that had taken place during development was obtained.

As on oat flour, the number of larvae developing to maturity was much higher with *O. mercator* than with *O. surinamensis*. Of the larvae of *O. mercator*, 13 became adults on each type of sugar, but with *O. surinamensis* the number of adults obtained was only three on each type of sugar. The difference in the development periods of the two species reported by Howe (*op. cit.*), and noted in the present work on oat flour, was magnified when the larvae developed on sugar. Whereas the difference on oat flour or wheatfeed is only about one day, on raw sugar the average development period of *O. mercator* was about 16 days longer than that of *O. surinamensis* and on yellow-crystal sugar it was about nine days longer. Although there was a greater variation in development times on raw sugar, larvae of both species were able to develop more rapidly on this than on yellow-crystal sugar (see Table III). For *O. mercator* the difference

TABLE III.

Larval development period in days, from hatching of egg to emergence of adult, on raw and yellow-crystal sugar.

	<i>O. surinamensis</i>		<i>O. mercator</i>	
	Raw sugar	Y.C. sugar	Raw sugar	Y.C. sugar
No. of adults emerged	3	3	13	13
Range in development time ..	39-41-51	57, 57-64	57-71	62-75
Average development time	43.7	59.3	59.9	68.6
Difference in development times (between sugars)	15.6		8.7	
Difference in development times (between spp.)	Raw sugar : 16.2 Y.C. sugar : 9.3			

of 8.7 days between the mean development times is significant ($P > 0.01$), for *O. surinamensis* the difference between the mean development time of the three larvae that became adult on each type of sugar is even greater, *viz.*, 15.6 days. Despite these indications that raw sugar is more suitable for the development of *Oryzaephilus* than yellow-crystal, it is of interest that most larvae of *O. surinamensis* that failed to complete their development on raw sugar died in the first instar, whereas on yellow-crystal sugar deaths were more uniformly spread among instars. With *O. mercator* most of the larvae that died did so in the third or subsequent instars on both types of sugar. The cause of death in many instances was the inability of the larva to moult successfully, but some larvae drowned in the syrup that formed in some tubes, especially in those containing *O. mercator*. The slower rate of development of this species meant

that a greater degree of deterioration could take place in the sugar before the larvae were full-grown and ready to pupate. This deterioration is characterised by the progressive solution of sugar crystals in the water that is taken up by the hygroscopic molasses film. The increasingly sticky environment that results appears to retard development and delay pupation and in consequence the ages of some of the larvae that were drowned were greater than the longest development period of those of the same species that became adult. The fate of the larvae of both species on the two types of sugar is shown in Table IV.

TABLE IV.

Fate of larvae of *Oryzaephilus* spp. developing on raw and yellow-crystal sugar.

Probable instar in which larva died					<i>O. surinamensis</i>		<i>O. mercator</i>	
					Raw sugar	Y.C. sugar	Raw sugar	Y.C. sugar
I	15 (2)	6 (1)	1	1
II	(2)	(3)	0	0
III	larva	..	0	1	[3]	[2]
			prepupa		0	4	1	1
IV (<i>mercator</i> only)			larva	..	—	—	0	1
			prepupa		—	—	0	1
Pupa	0	3 (2)	2 (1)	(1)
No. of adults emerged					3	3	13	13
Total					20	20	20	20

() indicates larvae died while moulting.

[] indicates larvae drowned in syrup.

Although a much higher proportion of larvae of *O. mercator* were able to complete their development on sugar, the ratios of the average development times on the two types of sugar to those on oat flour were lower for *O. surinamensis* than for *O. mercator* (Table V). Also, when the oviposition of three females of *O. surinamensis* derived from larvae reared wholly on sugar was observed on oat

TABLE V.

Ratio of average development periods of *Oryzaephilus* larvae on oat flour and on raw and yellow-crystal sugar.

	Oat flour	Raw sugar	Y.C. sugar	R./O.F.	Y.C./O.F.
<i>O. surinamensis</i> ..	24	43.7	59.3	1.84	2.47
<i>O. mercator</i> ..	25	59.9	68.6	2.40	2.74

flour the laying rate was actually higher than that observed previously on this material with females from larvae reared on the stock food (see fig. 1a). The total of six adults of *O. surinamensis* obtained from larvae reared on both types of sugar fortunately provided three pairs and it is from the oviposition of these that the comparison is drawn. In contrast, three paired females of *O. mercator* selected at random from among the 'sugar-reared' beetles and similarly tested and compared, showed a lower oviposition rate (see fig. 1b). No definite conclusions can be drawn from experiments involving so few beetles but there are indications in the above results that larvae of *O. surinamensis* are better able than those of *O. mercator* to develop on unrefined sugars. The much higher mortality, especially on raw sugar, of larvae of *O. surinamensis* in the early instars may be due to their smaller size and consequent lesser ability to contend with moist, sticky conditions that, at the start of the experiments, were more marked in raw than in yellow-crystal sugar. Fraenkel & Blewett (1943) state that *O. surinamensis* seems better fitted than other dried-food insects to live on a diet with a relatively high water content (they did not test *O. mercator*), but Thomas & Shepard (1940) found that on raisins at relative humidities above 70 per cent. young larvae frequently became caught in the sticky syrup produced and there was a high mortality.

Other observations.

It was possible to observe the oviposition of four 'sugar-reared' females of *O. mercator* (with males) on raw sugar. Two of the four pairs available were from each type of sugar but as the oviposition rates and total egg-production of the females were very similar they were treated as a common group. Egg-counts were made for six weeks after the pairing of the females. There was virtually no oviposition for the first two weeks, then each female laid sporadically and at a low rate during the next four weeks with two main periods of egg-production showing in each cycle. The average total of eggs for six weeks was 11, and the over-all viability 54 per cent. The average rate of oviposition on sugar for weeks three to six inclusive was slightly higher than during the same period for females that had been reared and matured on the stock food (see fig. 2). This may

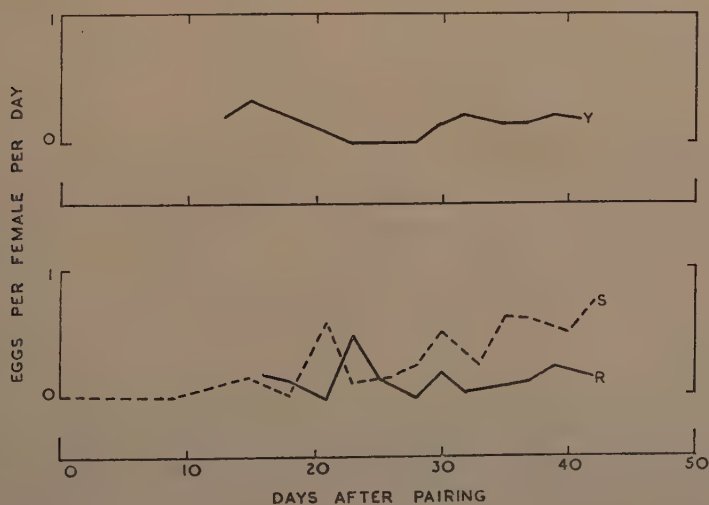


Fig. 2.—Oviposition rates of *Oryzaephilus mercator* on unrefined sugars. R, stock-reared females on raw sugar; Y, stock-reared females on yellow-crystal sugar; S, sugar-reared females on raw sugar.

indicate a degree of adaptation to the sugar diet, but the long preoviposition period and intermittent egg-laying suggest that some nutritional factor available in small measure in the molasses film must be accumulated over a period before a female can produce eggs.

The oviposition rate of 'stock-bred' females of both species that had been kept for six weeks since pairing on sugar quickly improved when they were transferred to oat flour, and this rate and the viability reached levels similar to those shown by females that had been on oat flour throughout. The average oviposition rates for the following three weeks of seven females (with males) transferred from raw and yellow-crystal sugar was compared with the rate over the same period for seven females that had been laying continuously on oat flour (see figs. 3a, 3b). The 'raw-sugar' beetles of each species showed a slightly better oviposition rate than those from yellow-crystal sugar, but whereas with *O. surinamensis* the rates for 'sugar' beetles were higher than for 'oat-flour' beetles, with *O. mercator* they were lower.

Recovery, in terms of egg-production, may therefore be expected to be rapid in both species when beetles reinfest cereal products after a short period on raw or yellow-crystal sugar. Females of *O. mercator* kept for over 200 days since pairing on either type of sugar were still able to lay viable eggs within a week of being placed on wholewheat flour.

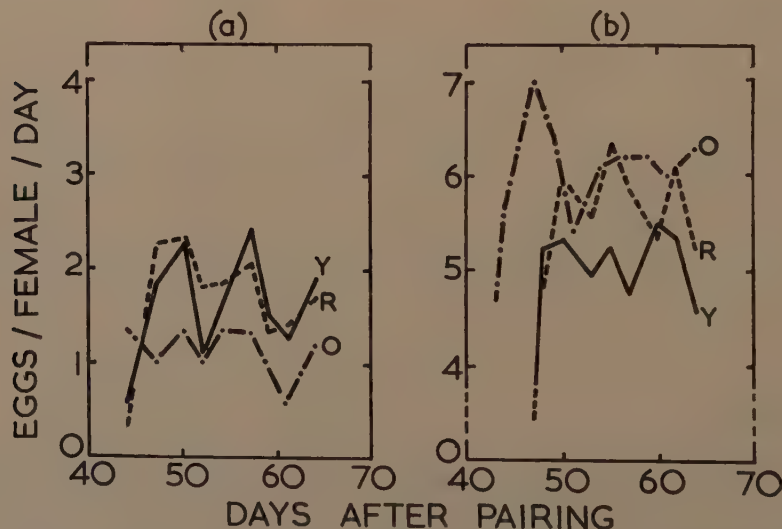


Fig. 3.—Oviposition rates of (a) *Oryzaephilus surinamensis* and (b) *O. mercator* on oat flour after six weeks on unrefined sugars. R, females transferred from raw sugar; Y, females transferred from yellow-crystal sugar; O, rate over the same period of females that had been laying continuously on oat flour. (From figs. 1a and 1b.)

Conclusions.

These results show that larvae of both *O. surinamensis* and *O. mercator* are able to complete their development on raw and yellow-crystal sugar. The part of the unrefined sugar actually eaten by the beetles is, of course, the molasses film on the surface of the crystals and there are indications that, as a species, *O. surinamensis* is better adapted to live on this food than *O. mercator*. Typical comparisons, however, could not be made because the Trinidad strain of *O. surinamensis* used in the experiments was smaller and apparently much less

vigorous than strains used by Howe (1956) and other workers. This lower vigour is particularly apparent in the oviposition rate of this species, which in all experiments was lower than that of *O. mercator*. Howe found that for at least the first month of the oviposition cycle, the laying rate of *O. surinamensis* was not less than twice that of *O. mercator*. The high mortality of larvae of *O. surinamensis* on raw and yellow-crystal sugar and the almost negligible oviposition of the females mean that despite a possibly better adaptation of this species to a 'sugar' diet, infestations of the strain considered here would eventually die out, even under favourable conditions. With *O. mercator*, however, about two-thirds of the larvae reared on sugar were able to complete their development and females derived from them laid sporadically on raw sugar, though at a very low rate, over the six-week period during which oviposition was observed. These survival and oviposition rates are sufficient to indicate that a population of this species could maintain itself on raw sugar although the rate of multiplication would be extremely low. It was not possible to test the oviposition of 'sugar-reared' females of *O. mercator* on yellow-crystal sugar, but 'stock-reared' females laid about 25 per cent. less eggs on this than on raw sugar. Assuming that 'sugar-reared' females will lay on yellow-crystal sugar and that a similar reduction applies, it is possible to calculate the weekly self-multiplicative rate of a population of *O. mercator* with a stable age distribution on both types of sugar. This is very low, being 1.05 on raw sugar and 1.034 on yellow-crystal sugar, and a population on each would take about 14 weeks and over 20 weeks, respectively, to double itself. The greater suitability of raw sugar for development is probably due to its higher molasses content (about 4 per cent. as against about 2.5-3 per cent. in yellow-crystal sugar) but there are also differences in the composition of the molasses films, for example the presence of titanium salts in yellow-crystal sugar. A factor which caused the death of some larvae developing on sugar under experimental conditions, namely, the accumulation of syrup in the tubes, would not operate to the same extent with sugar stored in stacked bags. This syrup results from the dilution of the molasses film, a process which as Breese & Wise (1959) have outlined, tends to be progressive in unrefined sugar stored in humid climates, especially in surface bags. Some of the syrup is absorbed in the fabric of the bags, but most of it that is 'free' drains slowly down through the stack and there is no serious accumulation except in the bottom bags. The longer development period of *O. mercator* would not therefore be such a disadvantage as experimental results suggest, except insofar as nutrients would become less available with the progressive leaching of the molasses film. As with *N. clavicornis*, the consequences of infestation (in terms of loss of sucrose) by *Oryzaephilus* spp. in raw or yellow-crystal sugar may be disregarded, but, in the humid tropics especially, the commodities must be taken into account when considering carry-over of infestation, or sources of infestation for cereal or similar products.

Summary.

Experiments were conducted with Trinidad strains of *Oryzaephilus surinamensis* (L.) and *O. mercator* (Fauv.), the average sizes of which were smaller than those given in the literature. Limited observations on their biology on cereal products showed that whereas the egg-production of *O. mercator* was comparable with that recorded by other workers under similar conditions, that of *O. surinamensis* was much lower.

Larvae of both species were able to complete their development on raw and yellow-crystal sugar, but there was a high mortality among those of *O. surinamensis*. Both species showed very low oviposition rates on sugar and this,

together with a high larval mortality, would indicate that infestations of *O. surinamensis* on these products would die out. *O. mercator*, on the other hand, could multiply very slowly. Oviposition rates rise rapidly when females return from sugar to cereal products.

Unrefined sugars should accordingly not be disregarded as sources of *Oryzaephilus* infestation, especially in the humid tropics.

Acknowledgement.

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DISCRIMINATIVE APPLICATION OF INSECTICIDE AGAINST *GLOSSINA MORSITANS* WESTW.

By K. S. HOCKING

Colonial Pesticides Research Unit, Arusha, Tanganyika.

Glossina morsitans Westw. is one of the savannah species of tsetse fly. It occurs in populations averaging from a few hundreds to a few thousands per square mile over many thousands of square miles of African savannah. To control such an insect by insecticidal means it is obviously desirable to be able to confine the application of insecticide to certain limited areas. Recent work on the perching places of savannah tsetse flies (Isherwood, 1957; Rennison, Lumsden & Webb, 1958; Jewell, 1958) has shown that during the day a large proportion of the flies is resting on the lower sides of horizontal branches of particular groups of trees. It was therefore decided to find out whether the application of a long-lasting insecticide to these resting places would eliminate tsetse flies from an isolated habitat.

Description of area.

The area chosen for this experiment, the Kabiganda Valley in south-western Uganda is a flat-floored valley, eight miles long and two to three miles wide, draining into the Kagera river near Kikigati on the Uganda-Tanganyika border. It is surrounded by steep, rocky hills, bare except for scattered examples of *Acacia hockii*. It contained about 17 square miles of mixed *Acacia* woodland infested with *Glossina morsitans*. Fly concentration areas were determined by searches for resting flies, supplemented by information from nearby fly-belts in similar woodland. Each fly concentration area consisted of one or more tall specimens of *A. gerrardii* associated with an understorey of *A. hockii* and young examples of *A. polyacantha* with one or more thickets made up of species of *Rhus* and *Grewia*. Game was plentiful, the commonest animals being buffalo, waterbuck, eland, wart-hog, duiker, bushbuck and reedbuck.

In 1955, the area was divided into six blocks, lettered A to F, each of approximately two square miles, and in each of these a pair of transect fly-paths was laid out. The fly-paths were numbered 1 to 12, and catching started in July 1955. (See map, fig. 1.)

Spraying operations.

The treatment consisted of a single application of a dieldrin emulsion spray (containing 3% (w/v) of dieldrin) to the lower sides of the branches of *A. gerrardii* up to a height of approximately ten ft. (or, in one area, to those of the associated thickets instead) by means of Eclipse knapsack sprayers fitted with extension lances and special nozzles. Spraying started in October 1957, and was completed in September 1958, except for a small area in the extreme north of the valley which was treated in November 1958. The periods of spraying each block were as follows:—

Block D	October–December 1957
Block E	January–March 1958
Block C	April–May 1958
Block B	June–mid-July 1958
Block A	Mid-July–mid-August 1958, and November 1958
Block F	Mid-August–end of September 1958.

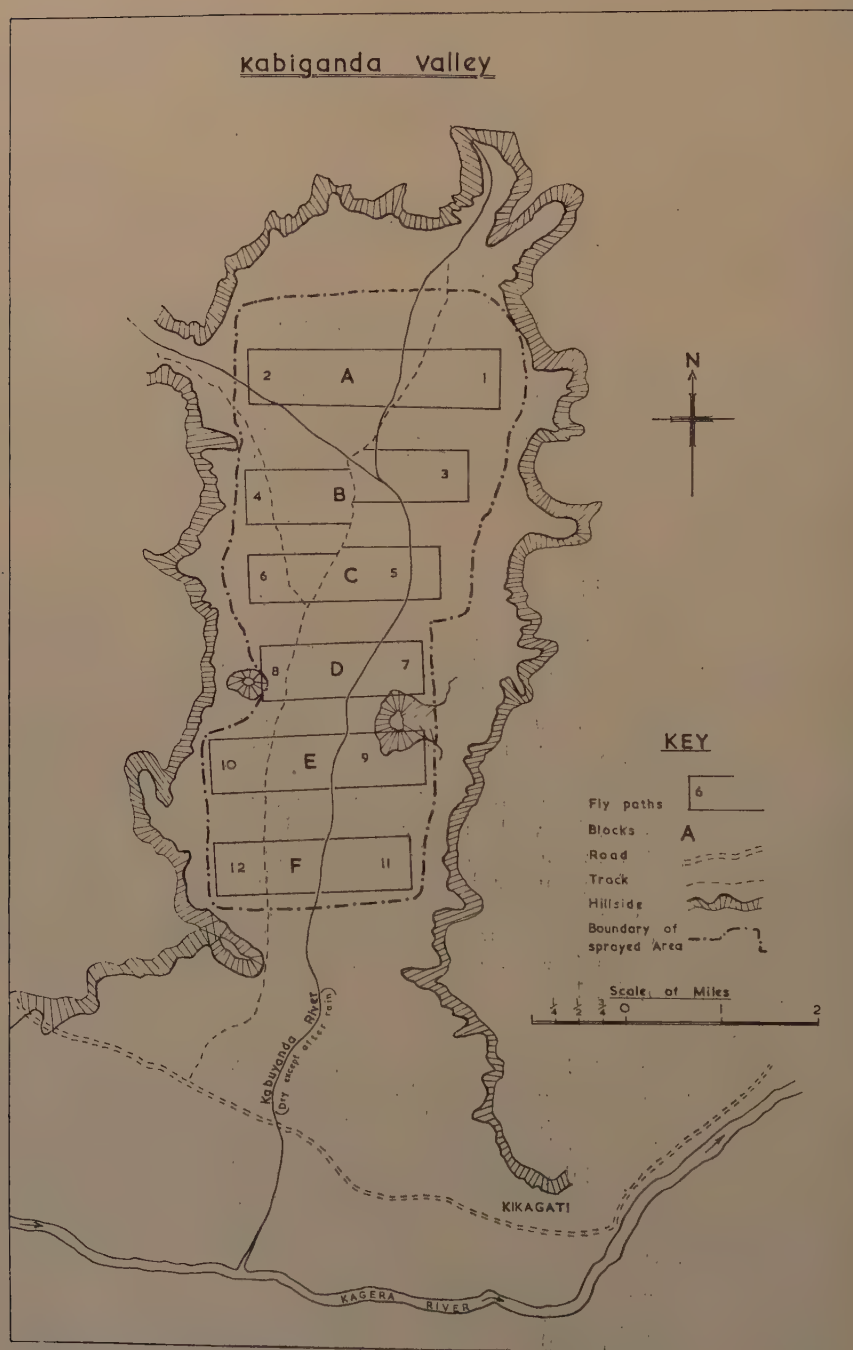


Fig. 1.—Map of the Kabiganda Valley showing the treated areas and the fly-paths.

After completion of the spraying of *A. gerrardii* in Block D in December 1957, the thickets associated with *A. gerrardii* in Block E were sprayed as a comparison. The spraying of the thickets was more costly in time and insecticide than the spraying of the acacias and did not appear to be any more effective; the rest of the valley was therefore treated by spraying *A. gerrardii* only.

Altogether about 11,000 acres of woodland were treated, and on an average 6.75 trees per acre, using altogether 1,300 gallons of Dieldrex, which contains 18 per cent. dieldrin (w/v) and was diluted to contain 3 per cent. dieldrin (w/v). This works out at approximately one-fifth of a pound of dieldrin per acre or approximately 75 cc. of Dieldrex per tree treated and it gave a deposit of approximately 0.8 g. of dieldrin per square metre on the surface sprayed.

Chemical estimations.

During the spraying of Block E, the persistence of dieldrin on the bark of *A. gerrardii* was estimated chemically. Using an emulsion spray containing 3 per cent. (w/v) of dieldrin, 20 trees were sprayed. Filter papers were used to obtain comparative measures of the initial deposits, and gave a mean deposit of 0.79 (S.D. ± 0.30) g. per sq. m. Bark samples, each of about 300 sq. cm., were removed at various time intervals up to 60 days after spraying. The surface deposits of insecticide were removed with light petroleum, and the insecticide that had been absorbed into the bark was then removed by extraction with hot petroleum for eight hours. Stepanow reduction to total chloride and Volhard titration were used for estimating the amounts of dieldrin.

TABLE I.

Apparent density of *Glossina morsitans* before and after spraying, derived from monthly catches on fly-paths.

	1955	1956	1957	1958	1959
January ..	—	80	(24)	25	1.0
February ..	—	80	—	22	1.2
March ..	—	69	—	24	1.3
April ..	—	27	(50)	27	0.8
May ..	—	48	(55)	19	0.8
June ..	—	60	—	24	0.9
July ..	63	39	36	10	0.3
August ..	34	37	37	3.3	0.18
September	29	18	35	2.1**	—
October ..	44	42	28*	2.5	—
November ..	48	44	19	0.7	—
December ..	61	41	23	1.2	—

* Month in which spraying was begun.

** Month in which spraying was completed.

Figures in brackets are those in which monthly catches were made on less than all the fly-paths.

There was evidence that a small proportion of the insecticide was absorbed into the bark soon after spraying. There appeared to be no further absorption, and loss thereafter seemed to be largely by reduction in the surface deposit. The decrease was very slight during the first five days, but thereafter appeared to be logarithmic with time. Expressing rates of deposits on the bark as percentages of the rates of deposits upon the paper samples taken during the spraying, the regression equation for the loss with time after the fifth day was:—

$$x = 85 - 36 \log_{10} (t-5)$$

where x is the deposit washed off the bark, expressed as a percentage of the initial deposit upon the sample papers, and t = time in days after spraying. This means that 90 per cent. of the insecticide disappears from the surface of the bark in about four months and 100 per cent. in about eight months. It is probable that 10 per cent. of the original deposit is quite sufficient to be lethal on long contact, and hence that the application remains effective for about four months.

Entomological results.

All the fly-paths were traversed by trained tsetse patrolmen on an average three times monthly and the apparent densities (numbers of non-teneral males per 10,000 yd.) of *Glossina morsitans* derived from each month's catches on these fly-paths from July 1955 until August 1959 are given in Table I. The mean monthly catches in each block (except F) before and after spraying, together with the mean catch from all areas that remained unsprayed, are shown in fig. 2.

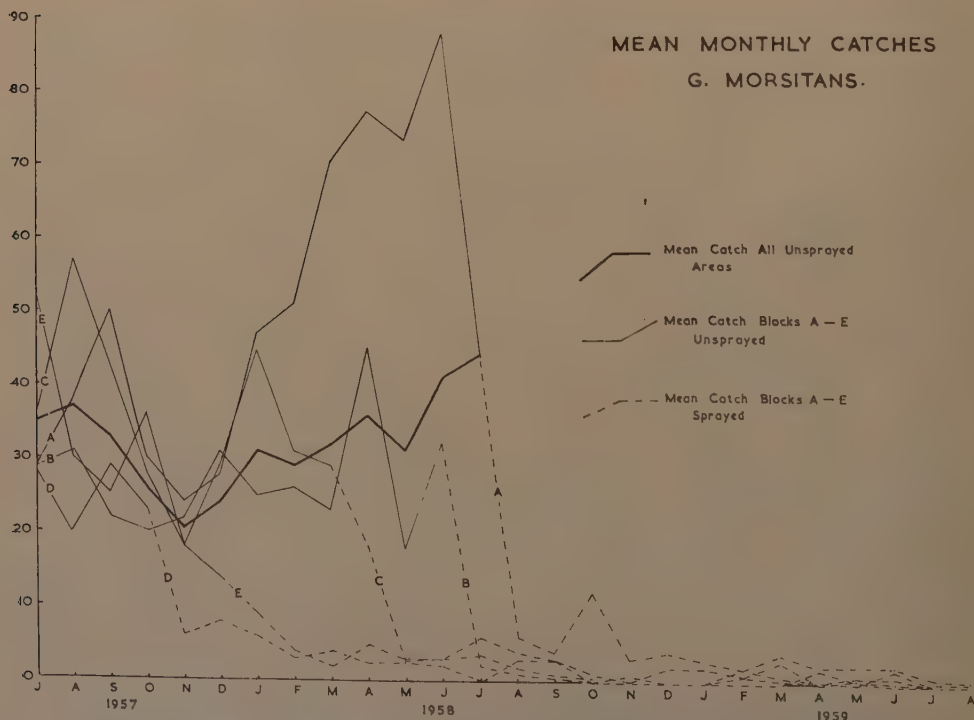


Fig. 2.—The mean monthly catches of *G. morsitans* from fly-paths in the Kabiganda Valley showing how the catches from each block fell off after the area was sprayed.

Block F is omitted from fig. 2 in order not to complicate this unduly. It will be seen that the catches fell greatly in each area after spraying and that the over-all reduction in the apparent density was over 99 per cent. Only three flies were caught in August 1959 on 70 miles of fly-path traversed compared with over 500 flies on a similar distance in August 1957.

Conclusions.

A very great reduction in the tsetse population of the valley was achieved, but small numbers still continued to be caught. It was thought possible that these flies were being brought in by the buffalo, which frequently came over the hills from neighbouring valleys, and hence that insecticidal applications of this sort if carried out over a wide enough area might eradicate a tsetse population.

Costs.

The costs of this experiment were as follows:—

	£
1,300 gal. Dieldrex @ Shs. 52/50 per gal.	3,400
Labour costs for spraying operations	500
Entomological staff	180
Transport costs	630
Salary of supervising field officer	840

The cost of the actual spraying operations, without salary of field officer and wages of entomological staff, was therefore about Shs. 8/- per acre, or about £250 per sq. mile.

Summary.

A dieldrin emulsion spray containing 3 per cent. dieldrin (w/v) was applied once to the putative resting places of *Glossina morsitans* Westw. throughout a comparatively isolated 17-sq.-mile fly-belt in the Kabiganda Valley in south-western Uganda between October 1957 and September 1958. Concentration areas of the fly consisted of one or more tall trees with associated understorey and thicket. The lower sides of the branches of an average of 6.75 such trees per acre were treated at a rate of about one-fifth of a pound of dieldrin per acre and at a cost of about £250 per sq. mile.

Chemical analysis showed a deposit of approximately 0.8g. dieldrin per sq. metre on the surface sprayed, and although 90 per cent. of this had disappeared from the surface after about four months, it is thought that the application remains effective for this period.

A very great reduction in the tsetse population was achieved. Small numbers of flies continued to be caught, but it was thought possible that these were being brought in from neighbouring valleys by buffalo, and, hence, that insecticidal application of this sort, if carried out over a wide enough area, might eradicate a tsetse population.

Acknowledgements.

My grateful thanks are due to Mr. A. G. Robertson, Director of Tsetse Control, Uganda, for his help and interest throughout the experiment. I would also like to thank Mr. A. Douglas Jones of Kikagati for his assistance and to congratulate Mr. T. H. Leather, Field Officer, on the able way in which he supervised the whole operation. The work was directed by the Colonial Pesticides Committee and financed from Colonial Development and Welfare Funds.

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LABORATORY OBSERVATIONS ON THE LIFE-HISTORY AND ETHOLOGY OF *MANSONIA* MOSQUITOS.

By J. D. GILLETT

East African Virus Research Institute, Entebbe, Uganda.

In October 1949, eggs were obtained from several species of *Mansonia* (*Coquillettidia*), and from one species of *M.* (*Mansonioides*), caught in and around Entebbe, Uganda. The eggs were allowed to hatch, and attempts were made to rear progenies through to the next generation. The present paper describes the rearing of a complete generation of *Mansonia* (*C.*) *aurites* (Theo.), and gives notes on the other species and on the genus in general.

It is well known that larvae and pupae of this genus attach themselves to the roots, stems and leaves of aquatic and semi-aquatic plants, larvae of *M.* (*C.*) *perturbans* (Wlk.) having been taken attached to the roots of emergent grass as long ago as 1907 by Dr. J. B. Smith in collaboration with Messrs. J. T. Brakeley and J. A. Grossbeck (Smith, 1908). Any attempt at rearing, therefore, must aim at finding a plant (or substitute) that is not only suitable for attachment, but remains in good condition for a sufficiently long period to allow completion of larval and pupal development. Ever since Mr. H. W. B. Moore found the larvae of *M.* (*Mansonia*) *titillans* (Wlk.) on the roots of the water-lettuce, *Pistia stratiotes*, in British Guiana (Dyar & Knab, 1910), and, independently, Ingram (1912) and Ingram & Macfie (1917) found those of *M.* (*Mansonioides*) *uniformis* (Theo.), and *M.* (*Mansonioides*) *africana* (Theo.), on the roots of *Pistia* in West Africa, and Dunn (1918) published his account of the importance of this plant in Panama, a tradition has grown up about *Pistia* and *Mansonia*. In Africa this tradition, which has particularly related to the subgenus *Mansonioides*, has not been supported by observation. Indeed, Ingram & Macfie (1917) themselves were careful to qualify their statement about this association by adding that no other plant had been incriminated by them *up to that time*. Since then there have been numerous instances recorded of larvae of *Mansonioides* being taken in Africa in the absence of *Pistia*, and on a wide variety of aquatic and semi-aquatic plants (Connal, 1928; Schwetz, 1930; Hopkins, 1936; Gillett, 1945, 1946). Furthermore, all the known larvae of the Ethiopian species of *Coquillettidia* have also been taken in the absence of this plant (Leeson, 1931; Wanson, 1944; Gillett, 1946; Wolfs, 1948*a, b*). Thus, although important, *Pistia* is only one of many plants utilised by the early stages of this genus. These long-recorded but apparently not so well-known facts have received further confirmation by the recent detailed work of Laurence (1959).

In searching for a suitable plant for laboratory rearing of *Mansonia* there is thus no reason whatever why *Pistia* should be first choice. Indeed *Pistia* is most unsuitable because it does not survive well in captivity and is too uneconomical of space to allow its use if larvae are to be isolated individually in separate tubes, an essential requirement when dealing with unidentified larvae.

Compared with mosquitos of other genera, the general metabolic pace in *Mansonia* would appear to be very slow: the larval mouth-brushes in all species studied move at only about 150 strokes/minute at 23°C., compared with about 350/minute in *Aedes* (*Stegomyia*) *aegypti* (L.) at the same temperature; locomotory movements of the larvae are also extremely slow; larval life in captive individuals of *M. aurites* takes 30–40 days, and pupal life in all species of

Coquillettidia studied varies from 6–11 days (depending on temperature). Thus, if plants are to be used they must remain in good condition for at least 6–7 weeks.

Wanson (1944) overcame this difficulty by using thick wrapping-paper, while Gillett (1946) used single, rooted stems of *Commelina* sp.* This remarkable plant not only develops a new and vigorous root-system within a few hours of submergence of the lower end of a severed stem, but it survives for many months, continuing to put out new roots and shoots all the time. Its long straight habit makes it particularly suitable if larvae are to be isolated in separate tubes.

Methods.

In the work to be described, both Wanson's and Gillett's methods were tried. As each egg-raft hatched, the young larvae were divided into two groups, one being provided with strips of special paper that had been sent to me by the late Professor Wanson, the other being placed in a glass dish of water containing several single stems of *Commelina*, each bearing a short length of root.

Eggs were obtained from wild-caught females of *M. (C.) metallica* (Theo.); *M. (C.) pseudoconopas* (Theo.), *M. (C.) maculipennis* (Theo.), *M. (C.) fusco-pennata* (Theo.), *M. (C.) aurites*, *M. (C.) fraseri* (Theo.), and *M. (Mansonioides) africana*. Nearly all these eggs hatched, but except for those of *M. aurites* that had been provided with *Commelina*, the first-instar larvae failed to attach themselves and died.

Life-history of *Mansonia aurites*.

Parent.

A blood-fed female of *M. aurites* caught in Lunyo Forest, near Entebbe, on 1st October 1949, was kept over water without access to any further blood. Eggs were laid 29 days later,† after which the adult died.

Eggs.

The eggs were laid in a long narrow canoe-shaped raft consisting of only four main rows of eggs of about 70 eggs in each, with a half row on either side. The eggs were yellow with an irregular black band half way up from the base (some eggs had two such bands) as described by Wanson (1944). They hatched after four days.

Larvae.

Newly hatched larvae fastened themselves to the roots of the plant within 24 hours of hatching, and were given daily increasing amounts of powdered dog-biscuit and Bemax as food. Under these conditions the first instar occupied 8–10 days, the second instar 6–10 days, and the third 9–14 days. Early fourth-instar larvae were shaken off the plants and isolated in separate test-tubes, each provided with a single, rooted stem of the plant. The larvae soon became anchored again, and renewed feeding. The fourth instar occupied 11–15 days, pupation taking place from 6th to 20th December 1949, 33–47 days after the eggs had hatched. Prepupae could be recognised 1–2 days before pupation. At each moult the larval pelts remained attached to the roots, except at pupation, when

* This plant is known as *nanda* locally, and was wrongly ascribed to *C. africana* by Gillett (1946). It now seems that more than one species is called *nanda*, the commonest being the blue-flowered *C. benghalensis*.

† This species feeds mainly on birds (Williams, Weitz & McClelland, 1958) and all specimens caught in the present series had already fed when captured. Thus, no exact data are available for the period between the blood-meal and oviposition. The period between capture of recently fed specimens and oviposition was usually six days, but under the conditions imposed on them this period was very variable—29 days in the complete life-history cited and 35 days in another.

they were 'kicked' off by violent circular movements of the pupal abdomen (Gillett, 1946). Considerable mortality occurred during larval life, and only 30 reached the pupal stage.

Pupae.

The pupae remained attached to the roots for 5-6 days. They then rose to the surface, leaving the pinnae embedded in the tissues of the plant. Some 12 hours later the adults emerged from the floating pupae.

Adults.

The first male adult emerged on 12th December, the last on 26th December, 1949; the first female emerged on 14th December and the last on 27th December. The adults were pinned and examined for variation in leg ornamentation. The range of variation usually encountered in this species has been described by Gillett (1949).^{*} It is interesting to note that this complete range was now shown in this progeny from a single female. Thus, of the 30 adults that emerged, the following forms occurred (17♂♂ and 13♀♀):

- Front tarsi:* 27 with 3rd, 4th and 5th segments all black. 3 "much more extensively yellow" (one pair being almost entirely yellow as in the type ♀ from Bonny, Nigeria).
- Middle tarsi:* 24 all yellow except for 5th segment. 6 with black tips to other segments.
- Hind tarsi:* 18 with 3rd segment all black. 12 with yellow base to 3rd segment.

General observations.

Smith (1956) refers to the eggs laid by a single female of *Mansonioides* as an egg-mass, no doubt in order to distinguish it from the more familiar floating raft of *Culex*. Laurence & Smith (1958) use a similar term in the same sense. The word mass has been used by Gillett (1959) to denote eggs laid by more than one female in a limited area, in contrast to a batch of eggs laid by a single female. In view of these differences in sense for the word mass, which in any case suggests a collection of indefinite shape, and hence more appropriate to the eggs laid by more than one female, the following terminology is proposed.

Batch of eggs (or *egg-batch*). The eggs laid by a single female mosquito following a single ovarian cycle (Gillett, 1955).

Cluster of eggs (or *egg-cluster*). A batch of eggs aggregated by cohesion. A cluster can be laid on the water surface and form a typical *egg-raft* as in *Culex* or *Coquillettidia*, or it can be laid on the underside of floating leaves as in *Mansonioides* (*la nacelle d'oeufs* of Schwetz, 1930).

Mass of eggs (or *egg-mass*). More than one batch of eggs (including clusters) in any given area.

Bates (1949) mentions that rafts of different groups of mosquitos tend to be characteristic in shape, and that those of *Mansonia* are usually long and narrow, consisting of only two rows of eggs. This has been found to be generally true in the Ethiopian species of *Coquillettidia*, except that the long canoe-shaped rafts usually consist of at least four rows of eggs. Now it is clear that differences in the shape of the raft must reflect differences in behaviour on the part of the ovipositing females, since Marshall (1938) has shown that shape depends on movements of the hind legs of the ovipositing female, rather than on properties of the eggs themselves. It is interesting to note, therefore, that *M. metallica*,

^{*} Edwards' (1941) description combines characters of *M. aurites* and *M. fraseri* and is, therefore, no longer valid.

others, the raft that comes nearest it in its l/w ratio is that of *M. maculipennis*. Thus, in both adult and larval characters, and in oviposition behaviour (as judged by egg-raft shape), it would seem justified to place *M. maculipennis* second on the list, immediately following *M. metallica*. The status of *M. annetti* is difficult to assess in the absence of larval and egg material.

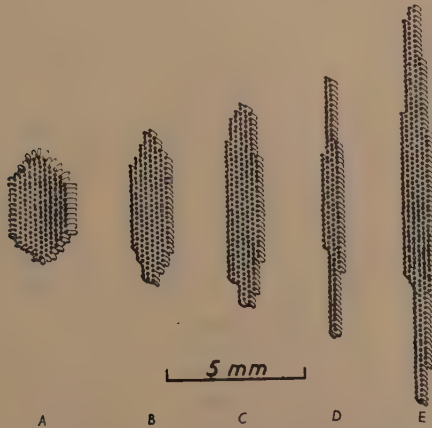


Fig. 1.—Egg-rafts of *Mansonia* (*Coquillettidia*) spp.: A, *M. metallica*; B, *M. maculipennis*; C, *M. fraseri*; D, *M. fuscopennata*; E, *M. aurites*. The difference between the boat-shaped raft of *M. fraseri* and the canoe-shaped raft of *M. aurites* should be noted. These two species had previously been separated on larval, pupal and adult characters (Gillett, 1949).

Whether or not such an arrangement reflects phylogenetic relationship is impossible to say. Edwards (1941), himself, has stated that ideally all stages of an insect's life-history should be taken into account in formulating a natural system, and the importance of larval characters in classification has more recently been emphasised by the late Dr. F. I. van Emden in a British Association symposium (Gordon, 1955). While it is clear that these authors had morphological characters in mind, I would go further and say that, where possible, behaviour should also be taken into account, whether or not any associated morphological differences (or likenesses) have been found, as both are ultimately determined at the biochemical level. The importance of feeding habits of biting flies in classification has recently been reviewed by Downes (1958). In suggesting this new arrangement I would emphasise that I am making no strong plea; I am merely putting it forward as a possible alternative in the light of our further knowledge of larval and oviposition characters. There may well be sound reasons for keeping the old arrangement, and it is wholly possible that my alternative violates accepted taxonomic principles. Nevertheless, if it serves only to clarify and strengthen the reasons underlying the current arrangement, it will at least have served a useful function.

At all stages in their life-history, species of at least the two Ethiopian subgenera show extraordinary similarities, even extending to apparently identical form of the male genitalia. This similarity in form may well have resulted from a restriction of evolutionary plasticity dictated by the requirements fitting them for their peculiar mode of life in the aquatic stages. Certain species, however,

notably *M. pseudoconopas*, *M. maculipennis* and the two species of *Mansonioides*, are highly variable in ornamentation, forms intermediate between species sometimes being almost as common as the typical forms. Although certain criteria can be used to separate *M. pseudoconopas* from *M. maculipennis* (Corbet, 1958) and the two species of *Mansonioides*, one is sometimes left with the impression that general similarity between species has allowed interspecific hybridisation to occur. Such hybridisation would, of course, normally be taken as evidence that we were dealing with divergence at the subspecific level.

Mention has been made of the general slow 'pace' in this genus. It seems probable that this general sluggishness is associated with the very specialised habits of the aquatic stages, when oxygen supply is probably low compared with that available to 'normal' larvae and pupae. It is not suggested that this is an example of cause and effect, but rather that a tendency to general slowness is an adaptation that makes it possible to live under these peculiar conditions. In other words, selection has operated in favour of a generally slow metabolism, which may well have been as important as the morphological modifications of the larva and pupa in allowing occupation of this ecological niche. This view receives support from the fact that slowness is not restricted to the aquatic stages; egg maturation in adults of *M. fuscopennata* at 23°C. takes seven days (Gillett, 1946; Haddow & Gillett, 1958).

Summary.

The rearing of a complete generation of *Mansonia* (*Coquillettidia*) *aurites* (Theo.) is described with notes on the methods used.

Variation in the leg ornamentation of the adults forming the progeny of a single egg-raft was found to cover the range usually observed in this species.

A plea is made for conformity in the use of terms describing the various kinds of aggregations of eggs.

Egg-rafts are discussed, and a tentative key is given to the eggs of six of the Ethiopian species of *Coquillettidia*, based on egg-raft shape.

A modification of the 'natural' arrangement is suggested for the Ethiopian species of *Coquillettidia*, based not only on adult and larval characters, but on oviposition behaviour as judged by egg-raft shape.

The possibility of natural interspecific hybridisation is mentioned.

The general slowness in many of the life processes found in the genus is put forward as an adaptation to its peculiar mode of life.

Acknowledgement.

It is a pleasure to be able to thank Miss Helen Sollers of the Plant Pest Control Division of the U.S. Department of Agriculture, Washington, D.C., for kindly arranging to send photocopies of the early American literature cited.

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THE USE OF PYRETHRUM FORMULATIONS TO CONTROL ANTESTIOPSIS ON COFFEE IN EAST AFRICA.

By T. J. CROWE

Coffee Research Station, Ministry of Agriculture, Kenya

G. D. GLYNNE JONES and RUTH WILLIAMSON

The Pyrethrum Board of Kenya Research Laboratories, Nakuru, Kenya.

Forms of *Antestiopsis lineaticollis* (Stål) *sensu lato*, and other species of *Antestiopsis*, are major pests of arabica coffee in East Africa (Le Pelley, 1959, pp. 54, 55). These Pentatomid bugs cause their most serious damage by infecting the developing coffee bean with the fungus *Nematospora coryli* (Wallace, 1931, 1932), but berry drop, flower abortion and distorted terminal growth can also result from their feeding (Le Pelley, 1942).

The material with which the present work was done comprised three distinct forms of *A. lineaticollis* of doubtful taxonomic status. No variation in response of any of the three forms to insecticides has been observed. Hereafter, the insect is referred to as *Antestiopsis*.

The average number of *Antestiopsis* per tree which causes economic damage varies according to the condition of the trees, their age, amount and value of the crop they are carrying, the percentage of *Antestiopsis* carrying *Nematospora* and numerous other factors. Le Pelley (1932) stated that, in Kenya, "It is certain that at 6 or 7 bugs to a tree, it is a major pest, causing notable loss. At 2 to a tree, it is probably advisable to employ control measures". Later, both Notley (1941), in Tanganyika, and Le Pelley (1942), in Kenya, attempted to correlate the average number of bugs per tree with the number of beans damaged by *Antestiopsis*. Both these attempts suggest that an economically dangerous level lies somewhere between 1 and 5 bugs to a tree. Progressive Kenya planters have for many years aimed at keeping populations below 2 to a tree and have consistently avoided serious damage. This evidence suggests that the target for a control measure should be to reduce the mean population to below 2 per tree.

If there are more than 20 bugs to a tree, it is almost always necessary to spray twice, due to the hatching of the unparasitised eggs not affected by the first spray. A practical control measure, therefore, is one that reduces the population by 90 per cent. at each treatment. Thus, a population of 20 or less should be reduced to 2 or under and a very high population of 100 reduced to 10, requiring a further treatment to effect the desired control.

The first successful use of pyrethrum against *Antestiopsis* was by Le Pelley (1932) in Kenya. The trees were covered by a cotton sheet and a hand atomiser was used to spray the trees with approximately $\frac{1}{2}$ fl. oz. of a kerosene extract of pyrethrum, obtained by extracting 1 lb. of ground pyrethrum flowers with 1 gal. kerosene. This suggests a pyrethrins concentration of approximately 0.1 per cent. w/v. A modification of this method using "double strength" extract, i.e., 2 lb. of flowers in 1 gal. kerosene, has since gained general acceptance for the purpose of assessing the populations of *Antestiopsis* and *Lygus* spp. on coffee trees.

Notley (1933) advocated the use of an aqueous emulsion of a kerosene extract of pyrethrum, used at the rate of about 2 pints per tree. This obviated the need for cotton sheets and allowed a better coverage of the foliage. Successes were

claimed, even though it is estimated that the pyrethrins concentration was of a low order, possibly only 0.002 per cent. w/v.

Notley (1936a) introduced a successful control method using finely ground fresh pyrethrum flowers dusted at the rate of 5–15 lb. per acre. This method was used by planters for many years, but gradually fell into disfavour due to a rise in the price of pyrethrum, the frequent variation between the performance of different batches and the loss of biological activity in storage.

Although pyrethrum extract was freely available from 1948 onwards, it is only recently that water-miscible concentrates have been marketed in East Africa. Similarly, dusts are now available containing a fixed percentage of pyrethrins* or synergised pyrethrins impregnated on a filler of standardised specifications.

The early work of Le Pelley and Notley, referred to above, established the high susceptibility of *Antestiopsis* to pyrethrum, and although this pest may equally well be controlled with other insecticides, such as DDT and malathion, there are considerable advantages accruing from the use of a highly selective insecticide, which does not persist on the foliage, when it is necessary to use chemicals as a supplement to normal biological control. The low mammalian toxicity of pyrethrum makes it ideal for use on such a valuable crop destined for human consumption; furthermore it is wholly safe in the hands of unskilled labour and peasant cultivators.

It is of interest to note that certain isolated areas under coffee in Kenya have never used insecticides other than pyrethrum to control *Antestiopsis*, and, in these areas, other pests, where they exist, rarely cause serious damage. Elsewhere, modern synthetic insecticides have been widely used, and insects which formerly caused negligible damage (notably the leaf miners, *Leucoptera meyricki* Ghesq. and *L. coffeina* Wshbn.) are now among the most important pests.

This paper describes laboratory and field experiments undertaken in Kenya to assess the value of certain modern pyrethrum formulations against *Antestiopsis*.

Materials.

Standard pyrethrum emulsifiable concentrate.

The following basic formulation was used throughout all laboratory and field trials.

Pyrethrum oleoresin (25% pyrethrins by P.B.K. Extract Method)	240 g.
Ethylan B.C.P. (non-ionic emulsifying agent)	300 g.
Commercial xylol added to make total volume	1000 ml.
Piperonyl butoxide when added replaced a portion of the xylol. This concentrate contained 6 per cent. pyrethrins w/v.	

Dusts.

The fillers used were a mixture of pyrethrum marc and limestone buffered to a pH of 5.9 and ground so that 98 per cent. passed through a B.S. 200 sieve. The marc was impregnated with pyrethrins before mixing with the limestone.

Laboratory experiments.

Determination of the value of adding a pyrethrum synergist.

The usefulness of pyrethrum as an insecticide has been considerably enhanced by the development of potent synergists such as piperonyl butoxide and sulfoxide. With many insect species, such as house-flies (*Musca domestica* L.), such synergists on addition to pyrethrum formulations at a pyrethrins:synergist ratio of 1:8 increase their potency five times or more, with an appreciable reduction in total

* The word 'pyrethrins', as used in this paper, is to be taken to include pyrethrins and cinerins.

cost. It is well known, however, that the action of a synergist is specific to a particular insect species and closely allied species may differ widely in their response. Tests were undertaken with *Antestiopsis* to discover if it responded to the presence of a synergist in a pyrethrum formulation.

Direct spray.—Groups of 20 adults of *Antestiopsis* were slightly anaesthetised with carbon dioxide and sprayed with a range of concentrations of pyrethrins with and without piperonyl butoxide in water emulsions in the laboratory spraying apparatus of Potter (1952). After treatment, the insects were transferred to glass jars containing green coffee berries and cotton-wool soaked with water, and were examined 24 hr. later. The dosage/mortality data in Table I show that the addition of piperonyl butoxide did not materially affect the kill.

TABLE I.

The effect of aqueous emulsion sprays of pyrethrins and pyrethrins + piperonyl butoxide on *Antestiopsis*.

Percentage concentration (w/v)		Percentage mortality in 24 hr.
Pyrethrins	Piperonyl butoxide	
0.001	—	90
0.001	0.001	100
0.001	0.005	95
0.001	0.008	90
0.0005	—	75
0.0005	0.0005	90
0.0005	0.0025	85
0.0005	0.0040	75
0.0001	—	20
0.0001	0.0001	25
0.0001	0.0005	10
—	—	Control 15

The volume of spray used in each test was 5 ml.

Residual films.—Using the same laboratory spraying apparatus, 12-cm.-square glass plates were sprayed with equal weights of a range of concentrations of pyrethrins in water-based emulsions (approx. 6.0 mg. aqueous emulsion spray per sq. cm.) with and without piperonyl butoxide. Six replicates were made with each concentration, and five adults of *Antestiopsis* were confined on two plates after these had been stored in diffuse daylight for 24, 48 and 72 hours, respectively. The mortality data in Table II fail to demonstrate that piperonyl butoxide was an active synergist for pyrethrum with *Antestiopsis* or that it imparted any substantial stability to the films.

TABLE II.

The effect of adding a pyrethrum synergist on residual films of pyrethrum.

Percentage concentration of spray (w/v)		Percentage mortality in 24 hr. when films stored for		
Pyrethrins	Piperonyl butoxide	24 hr.	48 hr.	72 hr.
0.001	—	65	40	20
0.001	0.008	80	15	20
0.0005	—	30	0	0
Controls	—	10	0	5

The relative effect of pyrethrum sprays on adults and nymphs.

Groups of ten adults and ten fourth- and fifth-instar nymphs were sprayed with four concentrations of pyrethrins as in the previous direct-spray test, but with half the volume of spray, and the mortality was assessed 24 hours later. This indicates that the nymphs were more resistant to pyrethrins than the adults (Table III). A parallel observation was made by Notley (1936b) who found that the fifth instar was more resistant to pyrethrum than the adult.

TABLE III.

The effect of an aqueous emulsion spray of pyrethrum extract on adults and nymphs.

Percentage concentration pyrethrins (w/v)	Percentage mortality in 24 hr.	
	Adults	Nymphs
0.005	100	100
0.0025	100	70
0.001	80	10
0.0005	45	6

The volume of spray used in each test was 2.5 ml.

Relative susceptibility of Antestiopsis and Musca domestica to pyrethrins.

Females of *Musca domestica* from a laboratory strain were found to have the same approximate body weight as adults of *Antestiopsis*, 0.03 g. per insect.

Toxicity tests were undertaken by topical application using the aqueous emulsion. The data obtained with *Antestiopsis* showed a considerable lack of uniformity in response and it was possible only to make an approximate evaluation of the LD₅₀ as 0.05 μ g.; that for *M. domestica* was 1.4 μ g., indicating the much greater susceptibility of *Antestiopsis*.

The rate of photolysis of pyrethrum on coffee leaves.

The factors affecting the rate of photolysis of pyrethrum in sunlight have been discussed by Glynne Jones (1960). A series of tests was undertaken with coffee leaves dipped in a range of concentrations of the aqueous pyrethrum spray used in the previous experiments and allowed to dry in the dark. Ten leaves were exposed to direct sunlight for 10 or 20 minutes and a further ten kept in the

TABLE IV.

The rate of photolysis of pyrethrum residues on coffee leaves subjected to bioassay with *Tribolium castaneum*.

Percentage concentration pyrethrins (w/v)	Exposure to sunlight (minutes)	Percentage <i>Tribolium</i> paralysed in 10 minutes	
		Leaves after exposure sunlight	Leaves from dark
0.77	10	56	100
0.77	20	10	100
0.10	10	12	100
0.05	10	18	95
0.05	20	0	100

dark as controls. Following this exposure, groups of ten adults of *Tribolium castaneum* (Hbst.) were confined within a metal ring on each leaf, and the numbers of paralysed insects recorded after ten minutes. The results summarised in Table IV reveal a rapid degradation in sunlight. It may be safely assumed that, in the field, coffee leaves sprayed with an emulsion containing 0.01 per cent. pyrethrins and exposed to direct sunlight would lose all residual toxicity in one hour. Leaves in the shade would probably require up to 24 hours.

Initial field trials.

Preliminary field trials were undertaken with a range of concentrations of the pyrethrum water-miscible formulation used at high volume (80–100 gals./acre). The results of counts made before and after spraying (Table V) show that only the highest concentration (0.018% pyrethrins) gave the required 90 per cent. control, and it appeared that the laboratory data were not applicable to field conditions.

TABLE V.

The effect of different concentrations of a pyrethrum water-miscible spray on a population of *Antestiopsis*.

Percentage concentration pyrethrins (w/v)	Mean no. <i>Antestiopsis</i> per tree	
	Before spraying	After spraying
0.006	15.1	5.3
0.008	28.1	8.6
0.01	9.3	1.6
0.018	20.6	1.1

A possible explanation for this discrepancy between laboratory and field results was that the response of the bugs to both lethal and sub-lethal doses of pyrethrum was rapid and uniform in that they all fell off the tree; but a proportion of them would not have picked up a lethal dose, probably because they did not receive a direct hit with a spray droplet. When these insects recovered, they returned to the tree, by which time a considerable proportion of the pyrethrum would have been destroyed by photolysis. Le Pelley (1934) had noted the recovery of a proportion of the insects knocked down by spraying with a kerosene extract of pyrethrum and suggested that children might be employed to collect the fallen bugs or that an additional gallon of spray should be applied to the ground after the bugs had fallen.

This hypothesis was tested as follows:

1. A coffee planter, Mr. T. K. Twist, reported very erratic results when using an 0.3 per cent. pyrethrins-impregnated dust, although a sample of this gave 100 per cent. kills when used in very small amounts in the laboratory. Using the standard assessment technique, kills of 20, 92 and 76 per cent. were claimed on various plots. He was advised to continue dusting as previously, but, 10–30 minutes after the application to the foliage, to place a few puffs of dust under each tree. High kills ranging from 83 to 100 per cent. were then obtained (average 96%).
2. Using a randomised-block layout, a more careful study was made of the effect of dusting the foliage together with either dusting under the tree or removing the bugs once they had been paralysed and fallen off the tree. Each 'plot' consisted of a single coffee tree from which all the bugs had been carefully

removed by hand. Each tree was infested with 12 fifth-instar nymphs taken from a laboratory culture. On the next day the following treatments were applied:

A : 15 g. of 0.3 per cent. pyrethrins dust applied to the foliage with a hand duster.

B : As A. In addition, 5 g. of dust was applied to the ground under the trees uniformly over a circular area equal in radius to the canopy of the tree.

C : As A. In addition, a chicken was tethered to the base of the tree with a foraging area beyond the canopy of the tree.

D : Untreated.

A barrier consisting of a strip of metal covered with banding grease was put on the ground around each tree about 6 ft. from the trunk to prevent the bugs walking across to another tree. (This proved to be unnecessary.) There were ten replications, making a total of 120 nymphs per treatment. Dead bugs were collected from the ground 9½ hours after treatment. Surviving bugs were collected from the trees the following day. Results are shown in Table VI.

TABLE VI.

The effect of different treatments under the tree following the application of a 0.3 per cent. pyrethrins dust to foliage.

Treatment	Total nymphs surviving on 10 trees	Percentage kill
A. Dust applied to foliage only	34	71.7
B. Dust applied to foliage and ground ..	15	87.5
C. Dust on foliage, chicken on ground ..	8	93.3
D. Control	113	5.8

Since the chickens were unable to eat nymphs until they were knocked down, the kill in treatment C gives the total knockdown. This was confirmed by observations on treatment A. Four to five hours after dusting, bugs were seen climbing back on to the tree. Some of these fell down again later, but about half the bugs recovering from knockdown survived the treatment completely. The results from these two experiments served to confirm the hypothesis that a proportion of the paralysed bugs was falling off the tree without having picked up a lethal dose.

Determination of minimum concentration of pyrethrins required to effect knock-down.

The previous experiments showed that it was necessary to consider the field control of *Antestiopsis* as a two-stage process, i.e., the removal of the insects from the tree, followed by action to prevent the ultimate survival of insects which had only received a sub-lethal dose and were capable of regaining the tree. In order to effect the first phase as economically as possible, experiments were undertaken to determine the minimum concentration of pyrethrins applied as a spray or dust which would effect 90 per cent. knockdown.

A similar layout with ten replications was used as in the previous experiment except that the trees, devoid of *Antestiopsis*, were infested with 20 fifth-instar nymphs. The spray was applied at the rate of 850 ml. per tree and dusts at 15 g. per tree. Thirty minutes after the application of toxicant the paralysed bugs on the ground around the trees were collected and a further collection was made

four hours later. The results (Table VII) show that to achieve 90 per cent. knockdown the minimum concentrations of pyrethrins required in sprays and dusts are 0.005 and 0.3 per cent., respectively.

TABLE VII.

Determination of minimum concentrations of pyrethrins, in sprays and dusts, required to effect 90 per cent. knockdown of fifth-instar nymphs of *Antestiopsis*.

Sprays		Dusts	
Percentage concentration pyrethrins (w/v)	Knockdown (%)	Percentage concentration pyrethrins (w/w)	Knockdown (%)
0.05	95.6	0.30	93.5
0.01	94.4	0.20	82.3
0.005	90.0	0.15	84.8
0.001	75.6	0.10	72.9
		0.05	64.7
S.E. ± 3.5		S.E. ± 3.5	

Application of a barrier to prevent return of bugs to tree.

Observations were made on the behaviour of bugs that had received sub-lethal doses of pyrethrins following the spraying of coffee trees. The majority tended to move to the centre of the tree within a few minutes of spraying, showing a characteristic pyrethrum-activation response. Then, either the bugs fell off the tree due to the onset of severe paralysis, or they walked unsteadily down the stem to the ground, often becoming paralysed at a later stage. Recovery occurred after periods ranging from a few minutes to several hours, and, although adults were able to fly for short distances, all stages of the bug regained the tree by walking up the stem. It is likely that ability to co-ordinate walking movements is regained before orientated flight is possible.

It was thought that the application of a persistent insecticidal dust at the lower part of the trunk of the tree (known hereafter as the bole), before or soon after the application of the foliage spray, would form the necessary barrier with which bugs would come into contact whilst descending or attempting to regain the tree. A 5 per cent. DDT dust, known to be toxic to all stages of *Antestiopsis*, was chosen, and tests showed that this material could be conveniently applied to the bole if contained in a small hessian sack which was banged on opposite sides of the trunk. This left a complete band of dust on the bark, and some dust was scattered on the ground near the trunk.

It seemed unlikely that such a localised application would have any appreciable effect on the parasites and predators of *Antestiopsis* or other insect pests. Furthermore, only one such dusting might be necessary even where two applications of spray to the foliage were required to control a heavy infestation.

A large-scale trial to confirm the value of a barrier of DDT dust was undertaken using plots consisting of 49 coffee trees arranged as a 3×3 Latin square. Five such squares were used, each laid out on a different coffee estate covering a range of vegetative growths and populations of *Antestiopsis*. It was hoped to determine whether the 5 per cent. DDT dust formed an effective barrier and to partition the contribution of the spray and dust to the total kill. The replicated treatments were (a) a 0.006 per cent. pyrethrins emulsion spray applied at 75 ± 5 p.s.i. until 'run-off' occurred, together with a 5 per cent. DDT dust applied to the bole just before spraying; (b) as (a) but without DDT dust; (c) untreated control. Each plot was separated from the next by a guard row of trees and wide hessian screens were used to prevent spray and dust drifts.

Three days after spraying, counts of bugs were undertaken, using the standard spray of kerosene extract of pyrethrum on a tented tree, on four trees selected at random from the inner 25 trees of each plot (edge effects were thus avoided). The results (Table VIII) confirm that the addition of the DDT dust had a significant effect on kill. The degree of control obtained on squares I and III was unsatisfactory and this was thought to be due to observed failure to effect proper spray cover within the dense foliage resulting from incorrect tree management.

TABLE VIII.

The effect of dusting the boles of the sprayed trees with a 5 per cent. DDT dust.

Square	Type of tree	Average volume of spray per tree (ml.)	Average weight of dust per tree (g.)	Average no. bugs per tree three days after spraying		
				Pyrethrins + DDT	Pyrethrins alone	Control
I	Short, single stem ; dense shell of foliage ; thin bole	565	4.8	3.00	5.83	15.33
II	Tall multistem ; little leaf ; very large knurled bole	810	13.1	0.33	1.00	5.75
III	Medium height multistem ; very leafy and badly pruned ; average bole	912	1.4	1.50	2.42	6.33
IV	Medium height ; average leaf ; average bole	918	3.5	0.125	0.50	2.25
V	Very tall ; multi-stem ; very leafy but well pruned ; average bole	1789	3.6	0.25	1.98	7.25
Average of all squares		999	5.3	1.07	2.17	7.39

The analyses of variance, using the results in Table VIII, showed that the differences between mean counts of *Antestiopsis* for each treatment were significant at the 1 per cent. level.

Further field trials.

Following the experimental development of a method of using the pyrethrum water-miscible concentrate, applied as a foliage spray, coupled with the application of a 5 per cent. DDT dust to the bole of the tree, further trials were undertaken on small plots of coffee, each of approximately 100 trees, belonging to members of the Kisii African Co-operative Society. In this area, it is desirable to combine the copper fungicide used to control the leaf rust, *Hemileia vastatrix*, with the toxicant used for *Antestiopsis* control as the absence of piped water and its general scarcity necessitates the minimum number of applications of foliage sprays. Preliminary laboratory experiments had shown that the proposed proprietary fungicide (Perenox) was fully compatible with the pyrethrum water-miscible concentrate at various dilutions. The trial was designed to test whether: (1) The

pyrethrum/DDT combination was an effective control for *Antestiopsis*; (2) The addition of the copper fungicide impaired the action of the pyrethrum; (3) A 0.5 per cent. dieldrin dust was superior to the 5 per cent. DDT dust.

Proper replication was not possible as the small coffee plantations were scattered over a wide area. Before spraying, counts were made of the population of *Antestiopsis* on ten trees chosen at random in each block. The spray was applied using a hand-operated stirrup pump with a no. 4 nozzle. Five days after each spraying, the population of *Antestiopsis* was estimated on 20 trees, and the treatment was repeated approximately 15 days after the first application. Half the trees of each block were treated with DDT on the bole and the other half with dieldrin. Each tree was sprayed to 'run-off' and although they varied in size the average rate of application was from $1\frac{1}{2}$ pints to 2 pints of spray per tree. The data obtained are summarised in Table IX. No data are given on the effect of each of the dust treatments as an examination of the counts from each half plot showed clearly that there were no significant differences between the populations of bugs on the dieldrin- and DDT-dusted trees.

TABLE IX.

Summary of data obtained from Kisii trials using foliage spray and DDT or dieldrin dust.

Treatment	Average no. bugs per tree			No. trees treated	Total spray used (gal.)
	Initial count	After 1st spray	After 2nd spray		
Pyrethrins w/v 0.005%	80	10	1	104	52
Pyrethrins w/v 0.005% + Perenox	34	9	1	100	48
Pyrethrins w/v 0.0075%	27	2	0	100	50
Pyrethrins w/v 0.0075 + Perenox	8	1	0	100	42
Ditto*	9	2	one spray only	75	18

DDT and dieldrin dusts used at rate of 1 lb. to 50 trees. Perenox used at 1 oz. per gallon spray.

* Experiment done at a different time.

The experiment showed that:

- (1) Where the population of bugs was less than 30 per tree, all treatments gave a satisfactory control following the first application. The second application gave satisfactory control of all the higher populations, even one averaging 80 per tree.
- (2) The addition of the fungicide did not impair the efficiency of the pyrethrins.

It should be noted that following the initial assessment of *Antestiopsis* on each plot, the ten trees used, although sprayed, had been freed from bugs and were excluded from the second and third counts. Similarly, the 20 trees used for the second count were given the second spray but were excluded from the trees used for the third and final count. It is unlikely, however, that this utilisation of 30 per cent. of the trees for counts introduced any particular bias into the experiments.

Commercial application of control method.

Approximately one thousand acres of coffee in the Kisii area were treated with spray containing 0.006 per cent. pyrethrins and the same proprietary fungicide, and the boles of the trees were treated with a 5 per cent. DDT dust. No special supervision was given and satisfactory control of *Antestiopsis* was reported by the local field officer.

Discussion.

The experimental evidence obtained has shown that when pyrethrum-based formulations are used to control *Antestiopsis* on coffee, the rapid paralytic action of this toxicant may operate adversely, resulting in insects leaving the site of application before acquiring a lethal dose.

The same phenomenon may well occur when this toxicant is used to control other phytophagous pests, and the subject will form the basis of further investigations.

The spray applications made were all at high volume and it is hoped to undertake trials using low-volume applicators.

Summary.

The history of the use of pyrethrum formulations to control *Antestiopsis* spp. on *arabica* coffee in Kenya is reviewed.

The material with which the present work was done comprised three distinct forms of *Antestiopsis* of doubtful taxonomic status; these were not observed to differ in their response to the insecticidal treatments used.

Laboratory experiments showed that *Antestiopsis* was highly susceptible to pyrethrum and that the addition of piperonyl butoxide, a pyrethrum synergist, did not affect this response.

Preliminary field experiments showed that concentrations of pyrethrins higher than those found effective in the laboratory were required. A hypothesis is advanced and established that this was due to a variable proportion of insects becoming paralysed and falling off the tree before acquiring a lethal dose.

These observations and experiments suggested a two-phase method of control, using pyrethrum as a non-persistent foliage spray at an economical but effective concentration of 0.005–0.006 per cent. pyrethrins, coupled with a 5 per cent. DDT or 0.5 per cent. dieldrin dust applied to the bole of the tree to form a persistent toxic barrier. The spray removed the bugs from the tree whilst the dust prevented the return of or killed those that had only received a sub-lethal dose of pyrethrum. It seemed unlikely that such a localised application of the persistent insecticide would have any appreciable effect on beneficial insects.

This two-phase treatment has been used successfully both in trials and in commercial practice for the control of *Antestiopsis*. When the initial population is in excess of 20 per tree, two spray applications and one dusting are necessary to effect control.

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We wish to acknowledge the use of data supplied by Mr. D. McCrae (Table V) and the helpful advice of Mr. J. Graham and Mrs. Elizabeth Green. We are grateful to the many coffee planters who have permitted experiments on their estates and to Mr. V. Burke and his staff of the Agricultural Department, Kisii, for their assistance with trials in that area. Messrs. Jaygee Products Ltd., Nairobi, kindly supplied some of the trial formulations.

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THE ATTRACTION OF MOSQUITOS BY HUMAN OR ANIMAL BAITS IN RELATION TO THE TRANSMISSION OF DISEASE.

By J. A. REID *

Institute for Medical Research, Kuala Lumpur, Malaya.

Comparisons between the number of mosquitos attracted to a bait of domestic animals and the number attracted to man have often helped to show up relatively anthropophilous species, among which disease vectors are likely to be found. Unfortunately, many investigators have not been content with the statement which their figures justify, that a particular species is *relatively* anthropophilous, compared with the other species present. They have tended to forget the other species, and have gone on to claim or imply that the species in question is anthropophilous in itself, which should mean that it is attracted in larger numbers to man than to animals, which it may not be. Wharton (1953) has shown how this has occurred with *Anopheles maculatus* Theo., the principal malaria vector of Malaya, and that it is not true for this species. He baited two window-trap huts with men or animals, and found that a hut with one calf caught slightly more individuals of *A. maculatus* than a hut with two men, but that the calf-baited hut caught 50 times more of the non-vector species, *A. philippinensis* Ludl., than the man-baited hut. That is to say, calf proved more attractive to *A. maculatus* than man, which was contrary to existing beliefs, but (and this is the important point) relative to *A. philippinensis*, *A. maculatus* was strongly anthropophilous. With *Culex pipiens fatigans* Wied., Wharton (1951b) caught larger numbers in the man-baited hut, but with *C. gelidus* Theo. he caught much larger numbers in the hut with the calf. It was decided to try to obtain similar information for other mosquito vectors of disease in Malaya, and the results are now recorded in this paper.

The names used for the mosquitos follow in the main the recent world list by Stone, Knight & Starcke (1959).

Methods.

Two mosquito-net traps were used in place of window-trap huts, which were not available; this proved an advantage in one respect because a number of species were caught which would probably not have entered a window-trap hut in significant numbers. The net traps were of the type that has been used for many years in Malaya (Gater, 1935, p. 80), consisting of a large mosquito-net, about 10 ft. long by seven ft. wide and high with a three-foot-wide gap in each side which can be closed by a flap whilst the mosquitos are being caught. The men who acted as the human bait were in one of these large nets, on camp beds more or less protected by small inner nets over their beds. The animals, one calf or two goats, were tethered inside the other large net which was some 20 to 50 yards away. At one place (site 3) the positions of the human and animal baits were switched over each week; unfortunately this could not be done at the other two sites. Each net was under a shelter without sides, and once an hour from 7 p.m. to 11 p.m. the flaps were lowered and the mosquitos caught, one man working in the animal-baited net, and one in the man-baited net. Usually, when catching finished at 11 p.m., the flaps were raised again and a further catch made at 5.30 a.m. the next morning, after which the catches were sent to the laboratory for identification. Possible disadvantages of this method, apart from the labour and human-error factors inseparable from the net-trap technique, were

* Now care of British Museum (Natural History).

the absence of one of the men from the man-baited trap for 10–15 minutes each hour to catch the mosquitos in the animal-baited trap, and the fact that, unlike the animals, the men were protected much of the time by the camp-bed nets; to this extent the trapping conditions in the man-baited nets were not the same as in the animal-baited nets.

Colless (1959a) has shown that even an unbaited net may catch a considerable number of mosquitos, but if this was a factor in the present experiments it would probably have affected both nets equally.

Areas.

Trapping mosquitos in such a way that the numbers of any species attracted to men and to animals can be directly compared, may, for ease of reference, be called comparative trapping. Comparative trapping with nets in the manner described was done in three different areas. In the first area (Rantau Panjang), referred to as site 1, *A. barbirostris* Wulp (dark-winged form, Reid, 1947) is the vector of human malaria, and Hodgkin (1956) had also found numerous plasmodial infections in *A. baezai* Gater, though there was a doubt whether these were of human origin. The area is a small, low-lying tongue of land between two tidal creeks fringed by mangrove forest, lying about four miles north-west of Klang town. It is shady and rather overgrown and there is a large and diverse mosquito fauna (some 70 to 80 species) breeding in ditches, earth wells, ground pools, crab holes, collections of water at the bases of nipah palms, fallen coconuts, etc. The small Malay population lives by making thatch from nipah-palm leaves, fishing, tapping rubber, or working in factories on the outskirts of Klang. There were no cattle at the time of the experiment and we had to bring in a calf of our own, but most households keep goats and we were able to borrow some of these. The people shut up their goats at night in small loosely constructed sheds raised off the ground like the houses, or tether them beneath the houses where the fowls are penned. Two experiments were made, the first in March–April 1952, the second in March 1953. Each experiment consisted of two trials, man *versus* calf followed by man *versus* goat.

The second area, Sungai Sirih (site 2), lies between Klang and Port Swettenham and is much like site 1 but is less shady, and perhaps more subject to inundation with brackish water from the Klang river. The malaria vector is *A. sudaicus* (Rdnw.) which, like *A. baezai*, breeds in brackish water. Three experiments were made, of which the second had to be discarded because walls had been erected on three sides of one of the shelters (a half-built house) used in the first experiment, and this greatly reduced the catch. For the third experiment we erected a temporary shelter of our own to replace this house. The first experiment was made in May–June 1952 and the third in April 1953.

The third area (site 3) is a lowland tea estate (estate B.C.) some 16 miles south of Klang, where Field and his colleagues conducted their classic experiment on atebirin prophylaxis before the last war (Field, Niven & Hodgkin, 1937). The vectors of malaria are *A. letifer* Sandosham and *A. umbrosus* (Theo.), breeding in or on the edge of the freshwater swamp forest growing on peat that surrounds the estate, which occupies a group of low laterite hills rising from the swamp. There is a large resident labour force concentrated in several groups of buildings. The site of the trapping experiment was some 50 yd. from the edge of one of these groups and between it and the forest edge about half a mile away. The labourers were mostly Southern Indians, and many owned cattle and goats which were kept at night in sheds grouped together at a point about 200 yd. away from the trapping site. One experiment, consisting of a single trial, was made during May–June 1957. The numbers of the malaria vectors were rather small, so that the man *versus* calf trial had to be continued for four weeks, and there was not time to test goats.

Results.

Site 1.

The combined results of the two experiments are shown in Table I. Only the main points need be mentioned at this stage. Taking the Anophelines first, it will be seen that the dark-winged form of *A. barbirostris*, the malaria vector, behaved very differently from the others; nearly four times as many were caught in the net baited with two men as were caught in the net with one calf, and nearly six times as many as in the net with two goats. For brevity, this (and similar data for other species) will be stated in the form that two men attracted nearly four times as many as one calf. But it must be remembered that this way of describing the results implies that the number of mosquitos attracted into the nets (which is not known) was the same as the number actually captured. More probably the number captured was smaller than the number attracted, but so long as the relationship remained constant the difference would not affect comparisons between the human and animal baits. However, the inner bed nets protecting the men may have caused more mosquitos to fly out again unfed from the man-baited nets than from the animal-baited ones. Conversely, some species may have stayed longer in the man-baited nets trying to reach the bait; this could conceivably explain the greater number of *A. barbirostris* captured with human bait. But, as will be seen later, the general picture which emerges from the results, and particularly the agreement with results produced by other methods (Table VII), does not suggest that the inner bed nets did cause much error. All the other Anophelines were attracted in much larger numbers to the animals than to the men; in consequence, *A. barbirostris* formed about 78 per cent. of the catches of Anophelines with human bait in both the man *versus* calf and man *versus* goat trials, but only two to three per cent. of those with animal bait.

Among the Culicines, also, the majority were caught in larger numbers in the animal-baited traps. However, there were a few species apparently more attracted to man, but the numbers were mostly rather small, though with *Aedes* (*Stegomyia*) *albopictus* (Skuse) and *Culex* (*Culex*) *p. fatigans* there are other reasons for thinking that the greater attraction to man was real. The commonest Culicines, those of which more than 100 specimens were caught with the calf, all showed a greater attraction to calf than to man, though in varying degrees. *Aë. 'butleri'* included several small black species of *Aëdes*, of which *Aë. (Aëdes) butleri* Theo. was by far the commonest, *Aë. (Skusea) amesii* (Ludl.) being next. *C. (Lophoceraomyia) 'fraudatrix'* may have included several species, for most of the females of this group could not be distinguished at that time, but one species which runs to *fraudatrix* Theo. in existing keys (though it is not this species; see Colless, 1960) appears to be much the commonest at Rantau Panjang. *C. gelidus*, *C. tritaeniorhynchus* Giles and *C. 'annulus'* together made up 40 per cent. of the Culicines caught with the calf; they belong to the banded-proboscis group of the sub-genus *Culex* and are common mosquitos in most inhabited areas; *C. 'annulus'* appears to have been mainly *C. annulus* Theo. with a smaller proportion of *C. pseudovishnui* Colless; prior to 1957 both were misidentified as *C. vishnui* Theo.; *C. tritaeniorhynchus* will have been subspecies *summorosus* Dyar (see Colless, 1957). *Mansonia* (*Mansonioides*) *'dives'* included both *M. dives* (Schiner) (= *longipalpis* (Wulp)) and *M. bonneae* Edw., which are very similar in habits as well as morphology; both are important vectors of filariasis due to *Brugia malayi* (= *Wuchereria malayi*, see Buckley, 1960) in certain other parts of the country. Though showing a slightly greater attraction to the calf than to the men, *M. 'dives'* was slightly more attracted to men than to goats.

Among the less common Culicines, one which should be mentioned because it occurred also at site 2 and appears to be relatively anthrophophilous (Table V),

is *C. (Culiciomyia) 'fragilis.'* It was probably a mixture of *C. fragilis* Ludl. and *C. spathifurca* (Edw.). It should be noted, however, that, in Singapore, Colless (1959b) found from precipitin tests that *C. spathifurca* was feeding on birds.

Considering all the mosquitos together, the grand totals show that the calf attracted about five times more mosquitos than two men, and two goats about twice as many. If Anophelines and Culicines are considered separately, the Anophelines were more attracted to the animals than the Culicines.

TABLE I.

Results of comparative trapping at site 1. Numbers of mosquitos caught.*

Species	14 nights		23 nights	
	2 men	1 calf	2 men	2 goats
<i>Anopheles</i>				
<i>baezai</i> Gater	5	89	2	41
<i>barbirostris</i> Wulp (dark-winged)	179	46	261	45
<i>kochi</i> Dön.	3	221	2	115
<i>lesteri</i> Baisas & Hu	42	1,733	69	988
<i>separatus</i> (Leic.)	0	57	1	62
<i>tessellatus</i> Theo.	0	54	0	20
<i>vagus</i> Dön.	0	236	0	6
Total Anophelines	229	2,436	335	1,277
<i>Aedes</i>				
<i>albopictus</i> (Skuse)	56	22	42	28
<i>aurantius</i> (Theo.)	5	31	2	26
' <i>butleri</i> '	336	397	461	732
<i>Armigeres</i>				
<i>malayi</i> (Theo.)	2	58	5	43
' <i>subalbatus</i> ' †	7	57	8	66
<i>Culex</i>				
<i>bिताeniorhynchus</i> Giles	6	31	12	14
<i>pipiens fatigans</i> Wied.	27	12	60	36
' <i>fragilis</i> '	26	11	25	28
' <i>fraudatrix</i> '	101	728	121	525
<i>gelidus</i> Theo.	49	644	78	134
<i>mimulus</i> Edw.	10	2	8	1
<i>nigropunctatus</i> Edw.	31	6	32	8
<i>tritaeniorhynchus</i> Giles	13	310	8	11
' <i>annulus</i> '	77	115	74	78
<i>Mansonia</i>				
' <i>dives</i> '	118	172	181	148
<i>uniformis</i> (Theo.)	8	35	26	24
<i>Uranotaenia</i> spp.	5	0	19	1
Total Culicines	877	2,631	1,162	1,903
Miscellaneous	8	16	13	9
Grand Total	1,114	5,083	1,510	3,189

* Only species of which ten or more individuals were caught with at least one of the baits are shown separately; the remainder, and unidentified species, are grouped under 'miscellaneous'.

† *Armigeres 'subalbatus'* was chiefly *Ar. subalbatus* (Coq.), referred to at the time as *Ar. obturbans* (Wlk.), probably mixed with small numbers of *Ar. kuchingensis* Edw. and *Ar. moultoni* Edw. (see Macdonald & Traub, 1960, p. 103).

Site 2.

The species and their relative numbers (Table II) were mostly similar to those at site 1. Among the Anophelines, the malaria vector, *A. sundaicus*, not present at site 1, was considerably more attracted by the calf than by the men, but the men were more attractive to it than the goats. However, it was much less markedly attracted to the calf than were the other Anophelines, for it formed

TABLE II.

Results of comparative trapping at site 2. Numbers of mosquitos caught.*

Species	14 nights		12 nights	
	2 men	1 calf	2 men	2 goats
<i>Anopheles</i>				
<i>aconitus</i> Dön.	1	10	0	0
<i>baezai</i> Gater	5	178	2	23
<i>barbirostris</i> Wulp (dark-winged)	0	7	11	1
<i>barbirostris</i> Wulp (light-winged)	11	178	3	24
<i>kochi</i> Dön.	9	615	6	69
<i>lesteri</i> Biasas & Hu	6	250	14	52
<i>sundaicus</i> (Rdnw.)	98	701	120	31
<i>tessellatus</i> Theo.	1	79	12	9
<i>vagus</i> Dön.	12	744	10	24
Total Anophelines	143	2,762	178	233
<i>Aedes</i>				
' <i>butleri</i> '	110	392	100	79
<i>Armigeres</i>				
' <i>subalbatus</i> '	0	10	1	0
<i>Culex</i>				
<i>pipiens fatigans</i> Wied.	381	349	630	212
' <i>fragilis</i> '	33	58	39	11
<i>fuscocephalus</i> Theo.	3	78	3	11
<i>gelidus</i> Theo.	143	2,191	90	325
<i>nigropunctatus</i> Edw.	5	10	3	12
<i>sitiens</i> Wied.	12	47	12	28
<i>tritaeniorhynchus</i> Giles	147	1,661	124	189
' <i>annulus</i> '	64	133	36	52
<i>Mansonia</i>				
<i>indiana</i> Edw.	11	18	20	7
' <i>dives</i> '	129	142	114	38
<i>uniformis</i> Theo.	48	193	24	35
Total Culicines	1,086	5,282	1,196	999
Miscellaneous	16	146	35	23
Grand Total	1,245	8,190	1,409	1,255

* Only species of which ten or more individuals were caught with at least one of the baits are shown separately; the remainder, and unidentified species, are grouped under 'miscellaneous'.

69 and 67 per cent. of the catches with human bait, compared with 25 and 13 per cent. of the catches with calf and with goats. The numbers of *A. barbirostris*, especially of the dark-winged form, were small, but the man versus goat trial suggests a considerable difference in the responses of the two forms.

Among the Culicines, the commonest species again included *Aedes* 'butleri,' *Culex gelidus*, *C. tritaeniorhynchus* and *C. 'annulus'*, and *Mansonia 'dives'*, with the addition of *C. p. fatigans* and *M. uniformis* (Theo.); *C. 'fraudatrix'* was absent. All except *C. p. fatigans* were again attracted in larger numbers to the calf than to the men, but this time *Aë. 'butleri'* as well as *M. 'dives'* showed more attraction to men than to goats, and the attraction of men for *M. 'dives'* was more marked than before. This may have been because the animal-baited trap had to be put in the same shelter as some goats which were penned at the other end, five to 10 yd. away. This probably reduced the catch when the trap was baited with two goats owing to competition from the other more numerous goats, but seems to have had the opposite effect when the calf was used. Presumably the penned goats, and the calf or two goats in the net trap, acted as a combined bait and attracted larger numbers of mosquitos to the vicinity than either alone would have done. The calf, being more attractive than goats, then 'captured' a large proportion of this enhanced number of mosquitos, resulting in a larger catch than expected. Certainly the grand total catches suggest that this is what happened, for they show that the calf attracted 6.6 times as many mosquitos as the two men, compared with 4.6 times as many at site 1, whilst the goats at site 2 attracted fewer mosquitos than the men, compared with twice as many at site 1. Again the Anophelines as a whole were more attracted to the animals than the Culicines.

Site 3.

Owing to the different environment of freshwater swamp forest growing on peat at site 3, instead of tidal mangrove forest as at sites 1 and 2, several of the species of mosquitos were different. The brackish-water species such as *A. baezai*, *A. sundaicus* and *Aë. 'butleri'* were absent, but species such as *A. letifer*, *A. separatus* (Leic.), *A. umbrosus*, *Aë. poecilus* (Theo.) and *M. annulata* Leic., which are associated with peat soil and swamp forest, were present. All the Anophelines were attracted in larger numbers to the calf than to the men, and this time the malaria vectors, *A. letifer* and *A. umbrosus*, did not form the majority of the Anophelines captured with human bait (Table III). Even when their numbers were added together they formed only 43 per cent. of this catch, 50 per cent. being due to *A. separatus*. However, whereas *A. separatus* formed 80 per cent. of the Anopheline catch with the calf, *A. letifer* and *A. umbrosus* together formed only 13 per cent., so that they can be said to have shown a greater attraction to man than did *A. separatus*.

Among the Culicines, *C. p. fatigans* was again attracted in larger numbers to the men, as was *Aë. (Finlaya) poecilus* Theo., but the numbers of the latter were small. The commonest Culicines were *C. p. fatigans*, *C. gelidus*, *C. tritaeniorhynchus*, *C. 'annulus'*, *M. 'dives'*, *M. uniformis* and *M. annulata*, of which all except the last named were also common at sites 1 or 2, or at both. All (except *C. p. fatigans*) were more attracted to the calf. Turning to the totals, the calf attracted about three times as many mosquitos as the two men, and once more the Anophelines were more attracted to the calf than the Culicines, about seven times compared with about twice.

Combined results from the three sites, and results from other countries.

Combined results.

A study of Tables I-III shows that most species which were common at all three sites, or at two of them, exhibited broadly the same pattern of attraction to the baits on each occasion. For example, at site 1 the number of individuals of *A. baezai* caught in the calf-baited trap was about 18 times more than the number caught in the man-baited trap, while at site 2 it was 36 times more. With *A.*

kochi Dön., at sites 1 and 2 these ratios were 74 and 68 to 1 in favour of calf, with *A. lesteri* Baisas & Hu 41 and 42 to 1, with *Mansonia* '*dives*' 1.5 and 1.1 to 1, and at site 3, 1.4 to 1. With *C. p. fatigans*, more were caught in the man-baited than in the calf-baited trap, the ratios at sites 1, 2 and 3 being 2.2:1, 1.1:1 and 2.9:1 in favour of man. In other words, within limits that are not unduly wide,

TABLE III.

Results of comparative trapping at site 3. Numbers of mosquitos caught.*

Species	20 nights	
	2 men	1 calf
<i>Anopheles</i>		
<i>indiensis</i> Theo.	6	36
<i>karwari</i> (James)	3	10
<i>letifer</i> Sandosham	38	60
<i>peditaeniatus</i> (Leic.)	2	23
<i>separatus</i> (Leic.)	126	1,485
<i>tessellatus</i> Theo.	8	50
<i>umbrosus</i> (Theo.)	70	173
Total Anophelines	253	1,837
<i>Aedes</i>		
<i>lineatopennis</i> (Ludl.)	3	17
<i>poecilus</i> Theo.	20	10
<i>vexans</i> (Mg.)	1	27
<i>Culex</i>		
<i>pipiens fatigans</i> Wied.	501	170
<i>gelidus</i> Theo.	496	2,493
<i>tritaeniorhynchus</i> Giles	29	77
' <i>annulus</i> '	25	105
<i>Mansonia</i>		
<i>annulata</i> Leic.	212	342
' <i>dives</i> '	183	262
<i>uniformis</i> (Theo.)	187	459
Total Culicines	1,657	3,962
Miscellaneous	11	40
Grand Total	1,921	5,839

* Only species of which ten or more individuals were caught with at least one of the baits are shown separately; the remainder, and unidentified species, are grouped under 'miscellaneous'.

the method has produced repeatable results with each species, so that it ought to be legitimate to treat the trials of man *versus* calf at the three sites as replicates of a single experiment and to add them together, and to treat the two trials of man *versus* goat similarly. This has been done in Table IV.

Some minor discrepancies between Table IV and Tables I-III will be noted; for example, in Tables I-III the light-winged form of *A. barbirostris* appears only in Table II, in which, in the man *versus* calf experiment, 11 were caught with human bait and 178 with calf. But, in Table IV, 185 are recorded as caught with calf; the additional seven were caught at site 3 but being less than 10 were placed in miscellaneous in Table III. The main point to notice in Table IV is the addition of columns showing the ratios between the numbers of each species

captured with human and with animal bait. These ratios make it plain that a calf is very attractive to most species, for only three (the dark-winged form of *A. barbirostris*, *Aë. albopictus* and *C. p. fatigans*) were caught in larger numbers in the man-baited trap. Goats were less attractive, and six species were caught in larger numbers with two men than with two goats, and this might have been increased to eight or nine species if goats had been used at site 3 where three

TABLE IV.

Combined results of comparative trapping at three different sites, and the ratios † between the numbers of mosquitos caught with human and animal baits.*

Species	48 nights			35 nights		
	2 men	1 calf	Ratio man : calf	2 men	2 goats	Ratio man : goat
<i>Anopheles</i>						
<i>baezai</i>	10	267	1 : 27	4	64	1 : 16
<i>barbirostris</i> (dark-winged)	179	53	3·4 : 1 ✓	272	46	5·9 : 1
<i>barbirostris</i> (light-winged)	11	185	1 : 17	3	24	1 : 8·0
<i>kochi</i>	12	837	1 : 70	8	184	1 : 23
<i>lesteri</i>	48	1,983	1 : 41	83	1,040	1 : 12
<i>letifer</i>	38	61	1 : 1·6	—	—	— : —
<i>separatus</i>	126	1,545	1 : 12	1	62	1 : 62
<i>sundaicus</i>	99	702	1 : 7·1	123	31	4·0 : 1
<i>tessellatus</i>	9	183	1 : 20	12	29	1 : 2·4
<i>umbrosus</i>	70	173	1 : 2·5	—	—	— : —
<i>vagus</i>	12	985	1 : 82 ✓	10	30	1 : 3·0
Total Anophelines ..	614	6,974	1 : 11	516	1,510	1 : 2·9
<i>Aedes</i>						
<i>albopictus</i>	56	22	2·5 : 1	46	28	1·6 : 1
'butleri'	446	789	1 : 1·8	561	811	1 : 1·4
<i>Armigeres</i>						
<i>malayi</i>	2	58	1 : 29	5	43	1 : 8·6
'subalbatus'	7	67	1 : 9·6	9	66	1 : 7·3
<i>Culex</i>						
<i>pipiens fatigans</i> ..	909	531	1·7 : 1	690	248	2·8 : 1
'fragilis'	59	69	1 : 1·2	64	39	1·6 : 1
'fraudatrix'	101	728	1 : 7·2	121	525	1 : 4·3
<i>fuscocephalus</i>	4	82	1 : 20	3	11	1 : 3·7
<i>gelidus</i>	688	5,828	1 : 7·8	168	459	1 : 2·7
<i>sitiens</i>	13	53	1 : 4·1	14	31	1 : 2·2
<i>tritaeniorhynchus</i> ..	189	2,048	1 : 11	132	200	1 : 1·5
'annulus'	166	353	1 : 2·1	110	130	1 : 1·2
<i>Mansonia</i>						
<i>annulata</i>	212	343	1 : 1·6	1	0	— : —
'dives'	430	576	1 : 1·3	295	186	1·6 : 1
<i>uniformis</i>	243	687	1 : 2·8	50	59	1 : 1·2
Total Culicines	3,525	11,734	1 : 3·3	2,269	2,836	1 : 1·2
Miscellaneous	141	404		134	98	
Grand Total	4,280	19,112	1 : 4·5	2,919	4,444	1 : 1·5

† Ratio figures over 10 are to the nearest whole number.

* Only species of which 50 or more individuals were caught with at least one of the baits are shown separately; the remainder are included in 'miscellaneous'.

species that did not occur at sites 1 or 2, namely, *A. letifer*, *A. umbrosus* and *M. annulata*, were not much more attracted to the calf than to the men. Even so, the majority of the species (17/23) were attracted in somewhat larger numbers to two goats than to two men, and the grand totals show that, adding numbers of all species together, the goats attracted 1.5 times more mosquitos than the men, while the calf attracted 4.5 times more than two men and about three times more than two goats.

Much more interesting, however, are the considerable differences between, for example, malaria vectors and non-vectors which the ratios bring out clearly. Thus, *A. barbirostris* (dark-winged form) and *A. sundaicus* are vectors of human malaria, whilst *A. kochi* and *A. vagus* Dön. are not, and the ratios show that two men attracted three times more individuals of the dark-winged form of *A. barbirostris* than one calf and six times more than two goats. In the case of *A. sundaicus*, the calf attracted seven times more than two men, though the men attracted four times more than the goats; possibly, in view of the effect of the other goats near the animal-baited trap at site 2, these ratios should be corrected to about 5:1 in favour of calf over men, and 2:1 in favour of men over goats (see p. 48). In the case of *A. kochi*, the calf attracted 70 times more than the men, and the goats 23 times more, and the corresponding figures for *A. vagus* were 82 times and three times more than the men. It looks as if these ratios may be a useful way of comparing the degree of attraction of different species to men or animals. This comparison has been attempted in Table V, in which the species are arranged according to these ratios in order of increasing attraction to calf (reading from the top of the table downwards).

It is not suggested that the actual values of the ratios (which might be called attraction ratios) are of great importance, for obviously these values are liable to variation from several causes; for example, mere sampling errors. Thus, Table IV shows that the number of individuals of *A. baezai* caught with human bait was small (10), and the addition of five to this and to the number caught with calf would have lowered the ratio from 1:27 in favour of calf (10/267) to 1:18 (15/272). Another cause of variation would be the size or species of animal; if a full-grown cow had been used instead of a calf, the ratios in favour of the animal would probably have been distinctly larger, and we have seen that with goats the ratios are smaller; or if a different kind of trap had been used with narrower entrances than in the nets, e.g., a window-trap hut, the ratios would have been different (see Table VII). What is suggested is that though the values of the ratios would be different if the experimental conditions were changed, the order in which the species would be placed by their ratios would be broadly the same. This suggestion can be tested in two ways. The ratios for species for which data are available for both net traps and window-trap huts are compared in Table VII, and it will be seen that though the ratios for each species from the two kinds of traps differ markedly, the order in which the species are placed by these ratios is exactly the same. The second test consists of arranging the man:goat ratios in Table IV in order, and comparing the species order so obtained with that in Table V based on the man:calf ratios; the order is roughly the same.* Nine out of the first ten species are the same in both lists which both begin with the dark-winged form of *A. barbirostris*. (*A. letifer*, *M. annulata* and *A. umbrosus* must be omitted from the first ten in Table V as they were not present in the man:goat trials, *A. sundaicus* is then the tenth species.) Except for *A. sundaicus* moving from tenth place in the man:calf ratios to second in the man:goat ratios, the

* The species order based on the man:goat ratios is: *Anopheles barbirostris* (dark-winged), *A. sundaicus*, *C. fatigans*, *Aedes albopictus*, *C. 'fragilis'*, *M. 'dives'*, *C. 'annulus'*, *M. uniformis*, *Aë. 'butleri'*, *C. tritaeniorhynchus*, *C. sitiens* Wied., *A. tessellatus* Theo., *C. gelidus*, *A. vagus*, *C. fuscicephalus* Theo., *C. 'fraudatrix'*, *Armigeres 'subalbatus'*, *Anopheles barbirostris* (light-winged), *Armigeres malayi* (Theo.), *Anopheles lesteri*, *A. baezai*, *A. kochi*, *A. separatus*.

TABLE V.

The species arranged in their order of attraction to calf or man, starting with those more attracted to man, with an indication of their status as vectors of human disease in Malaya.

Species	Ratio man : calf	Ratio groups	Proportion of disease vectors in the ratio groups	Vector of		
				Malaria	Filariasis	Dengue or Japanese B encephalitis
<i>Anopheles barbirostris</i> (dark-winged)						
<i>Aedes albopictus</i> ..	3:4 : 1	>1:1 to about 1:1 (two men attracted more mosquitos than one calf, or about as many)	4/5	+	47 5 + 8 mosquitos	—
<i>Culex pipiens fatigans</i> ..	2:5 : 1			—	29 8 + 10 mosquitos	+
<i>Culex 'fragilis', ..</i>	1:7 : 1			—	—	—
<i>Culex 'fragilis', ..</i>	1 : 1:2			—	—	—
<i>Mansonia 'daves', ..</i>	1 : 1:3			—	62 30 + 10 mosquitos	—
<i>Anopheles letifer</i> ..	1 : 1:6	1:2 to 1:5 (one calf attracted from about twice to five times as many mosquitos as two men)	5/7	+	44 17 + 10 mosquitos	—
<i>Mansonia annulata</i> ..	1 : 1:6			—	—	—
<i>Aedes 'butleri', ..</i>	1 : 1:8			—	—	+
<i>Culex 'annulus', ..</i>	1 : 2:1			—	—	—
<i>Anopheles umbrosus</i> ..	1 : 2:5			+	—	—
<i>Mansonia uniformis</i> ..	1 : 2:8			—	47 5 + 15 mosquitos	+
<i>Culex sitiens</i> ..	1 : 4:1			—	—	—
<i>Anopheles sundaticus</i> ..	1 : 7:1			+	—	—
<i>Culex 'fraudatrix', ..</i>	1 : 7:2			—	—	+
<i>Culex gelidus</i> ..	1 : 7:8	1:6 to 1:15	3/6	—	—	—
<i>Armigeres 'subulatus', ..</i>	1 : 9:6			—	—	+
<i>Culex tritaeniorhynchus</i> ..	1 : 11*			—	—	—
<i>Anopheles separatus</i> ..	1 : 12			—	—	—
<i>Anopheles barbirostris</i> (light-winged)						
<i>Anopheles tessellatus</i> ..	1 : 17	1:16 or more	0/8	—	—	—
<i>Culex fuscocephalus</i> ..	1 : 20			—	—	—
<i>Anopheles baезаи</i> ..	1 : 20			—	—	—
<i>Armigeres malayi</i> ..	1 : 27			—	—	—
<i>Armigeres malayi</i> ..	1 : 29			—	—	—
<i>Anopheles lesteri</i> ..	1 : 41			—	—	—
<i>Anopheles kochi</i> ..	1 : 41			—	—	—
<i>Anopheles kochi</i> ..	1 : 70			—	—	—
<i>Anopheles vagus</i> ..	1 : 82			—	—	—

* Ratio figures over 10 are to the nearest whole number.

species orders within the first ten of both lists are not very different. Similarly, for the last ten species (the most zoophilous), eight are the same in both lists. Thus, using a different type of trap did considerably alter the values of the attraction ratios, but made no difference to the order in which the species were placed by these ratios. Even using a different bait, goat instead of calf, did not make much difference to the species order, though possibly if some very different bait had been used, such as birds, the order might have changed considerably.

So it seems that the species order in Table V may have more than a purely local significance limited to these experiments. To test this further, the species which are known to be vectors of human disease in Malaya are indicated by + signs in Table V, and it will be seen that there are more of these signs towards the top of the table where the less zoophilous species are placed. To summarise this information and make it easier to analyse, the range of attraction ratios has been divided into arbitrary groups (ratio groups, third column in Table V), and one sees that there is a close correlation between these groups and the number of vectors of human disease in them (fourth column). In the first group, containing five species for which a bait of two men was more attractive than one calf or about equally attractive, four out of five are disease vectors (five out of six if *A. maculatus* is added—see below). In the last group, where the calf attracted 16 or more times as many of each species as two men, there are no proved vectors. If there were still any doubt that the order in which the species are placed by their attraction ratios is significant, this should dispel it.

To avoid confusion, a standard method of writing the attraction ratios has been used in the present paper. Man appears on the left of the ratio and animal on the right; then if the figure on the left is greater than unity this indicates a species more attracted to man than to animal, and *vice versa*. Thus, in Table V, the man:calf attraction ratio for *Aē. albopictus* was 2.5:1 (preference for man), while for *A. umbrosus* it was 1:2.5 (preference for calf).

Vectors of malaria.

Before discussing these, it is desirable to try to fit *A. maculatus*, the principal malaria vector of Malaya, into the picture. This can be attempted by making use of Wharton's figures from his experiments with window-trap huts (Wharton, 1951a). Combining the relevant figures from his Tables I, III and IV there were 405 individuals of *A. maculatus* caught in the huts baited with men compared with 532 in the hut baited with a calf, giving a man:calf ratio of 1:1.3. This would place *A. maculatus* between *C. p. fatigans* and *C. 'annulus'* in Table VII of the present paper, and reference back from there to Table V shows five species between *C. p. fatigans* and *'annulus'*. Presumably *A. maculatus* would fall somewhere among these five, either near the bottom of the first ratio group or the top of the second. Thus, under the conditions of these experiments, the man:calf attraction ratios of the Malayan vectors of malaria seem to range from about 3:1 in favour of man for the dark-winged form of *A. barbirostris*, through *A. maculatus*, *A. letifer* and *A. umbrosus*, to 1:7 (or perhaps 1:5, see p. 51) in favour of calf for *A. sundaicus*.

On the whole, this order agrees well with the vector status of these species (Table 47 in Hodgkin, 1956); the only surprise is to find the dark-winged form of *barbirostris* more anthropophilous than *A. maculatus*, which is the more important vector in Malaya, but this seems to be confirmed by the results of precipitin tests. In a small series of 29 dark-winged *barbirostris* caught blood-fed in their outdoor day resting places at site 1, there were 27 positive reactions, and, of these, 17 (over 60%) were for human blood; this compares with 10–20 per cent. in *A. maculatus* (Wharton, 1953). There may be several reasons why *A. maculatus* is, nevertheless, the more important vector; one is that it is more widespread, it occurs in all cultivated hilly land and has, therefore, long been known as the major vector on

the rubber estates. The dark-winged form of *A. barbirostris* is largely confined to certain parts of the coastal plains, mainly on the west side of the peninsula, many of which are occupied by peasant farms rather than by estates. Largely for this reason its importance as a vector has only slowly been realised. Also it may be a less favourable host for the human malaria parasites than *A. maculatus*; it is certainly difficult to infect with *Plasmodium falciparum* compared with *A. maculatus* (Hodgkin, 1950, p. 327).

The non-vector Anophelines were all more zoophilous than the malaria vectors, their man: calf attraction ratios ranging from 1:12 for *A. separatus* to 1:82 for *A. vagus* (Table V).

Vectors of filariasis.

In Malaya, filariasis is predominantly a rural disease due to *Brugia malayi*. Only a few small foci of filariasis due to *Wuchereria bancrofti* are known at present; in one of these, in urban Singapore, Danaraj, Schacher & Colless (1958) found *C. p. fatigans* to be infected; in a rural focus, on the Pahang river, Wharton (1960) found that a species close to *A. letifer* was the vector.

Like the malaria vectors, the vectors of filariasis in Table V are among the less zoophilous species. Until recently, *A. lesteri*, in the *hyrcanus* group (man: calf ratio 1:41) was thought to be a vector of *B. malayi* (Reid, 1955, p. 79). However, re-examination by Dr. Wharton of the mature filarial larvae from *A. lesteri* showed that, despite a close resemblance, they are not *B. malayi*, but possibly a species of *Setaria*, presumably from cattle.

Vectors of dengue or Japanese B encephalitis.

The epidemiology of these and related virus diseases is only partly understood as yet. There appear to be animal reservoirs of some of the infections. *Aë. albopictus* is included in Table V because it is usually accepted as a vector of dengue on epidemiological grounds, and is an effective laboratory vector. The remaining species marked as virus vectors in Table V are those from which the virus of Japanese B encephalitis has been isolated by our American colleagues working at this Institute (Traub, 1957, p. 105). As with the vectors of malaria and filariasis, these virus vectors are all among the less strongly zoophilous species, though the two most important vectors of virus of Japanese B encephalitis, *C. gelidus* and *C. tritaeniorhynchus*, are more zoophilous than the malaria vectors. This might be expected since Japanese B encephalitis appears to be primarily an infection of animals (see next paragraph).

Japanese B encephalitis is an infection, often inapparent, that is common both in man and domestic animals in Malaya (Pond & others, 1954) in which the animals rather than man are thought to be the reservoir. Thus, the finding of the virus in a particular species of mosquito is not by itself proof that that species is a vector to man. Additional evidence, such as that presented here, showing that the species is common enough, and has sufficient contact with both animals and man, to act as a vector between the two, may therefore be helpful. The very brief period during which the mammalian host is usually infectious to mosquitos, followed as it is by immunity (Hale, Lim & Colless, 1957), means that the sources of virus at any particular moment (animals circulating virus in the blood) from which mosquitos can acquire the infection, will usually be few and widely scattered. This would seem to require a vector both numerous and long-lived to keep the infection going, and species such as *C. tritaeniorhynchus* and *C. gelidus* seem to fit these requirements rather well. Both are very common, and there is evidence that both are quite long-lived. Newson & Blakeslee (1957), reporting on a laboratory colony of the Japanese form of *C. tritaeniorhynchus*, found the

maximum length of life for active adult females was over 100 days, and Reid (1958, p. 81) records an average adult life of about 4-6 weeks for *C. gelidus* in a laboratory colony. However, Colless (1959b) is inclined to doubt whether these species have enough contact with man and a high enough infection rate to be the main vectors of the disease to man in Malaya. He favours species of *Armigeres* and *Mansonia*, and it will be seen from Table V that *M. uniformis* has been found infected.

Wider application of the attraction ratio; results from other countries.

Arranging the species in order by their attraction ratios, as was done in Table V, proves to be useful in considering their relation to disease transmission. Accordingly an attempt has been made in Table VI to apply the method to

TABLE VI.

Attraction ratios calculated from comparative trapping results published by various authors.

Species	No. caught (actual or calculated) on			Ratios
	Man	Cow	Goat	
<i>Anopheles</i>				man : cow
<i>funestus</i> ¹	259	5	—	52 : 1
<i>funestus</i> ²	74	3	—	25 : 1
<i>gambiae</i> ¹	152	30	—	5.0 : 1
<i>gambiae</i> ²	178	23	—	7.7 : 1
<i>darlingi</i> ³	140	42	—	3.3 : 1
<i>pseudopunctipennis</i> ⁴	410	545	—	1 : 1.3
<i>coustani</i> ¹	3	29	—	1 : 9.7
<i>aquasalis</i> ⁵	31*	345*	—	1 : 11
<i>pessoai</i> etc. ³	53	600	—	1 : 11
<i>Culex</i> ⁶				man : goat
<i>pipiens pallens</i>	385	—	100	3.8 : 1
<i>iritaeniorhynchus</i>	1,210	—	2,840	1 : 2.3
<i>vishnui</i>	10	—	50	1 : 5.0

* Daily average catch over seven and a half months.

1, Haddow (1942); 2, Smith (1955); 3, de Zulueta (1952); 4, Sasse & Hackett (1950); 5, Senior White (1952); 6, Sasa & Sabin (1950).

suitable published data from other countries; the results are encouraging. In this table, only the man *versus* cow trials of the various authors have been included, except for some results with Culicines in which goat but not cow, was used. Where the number of nights catching (trials) with the two baits was not the same, the average nightly catch has been used to make a correction; thus, de Zulueta made six trials with man and four with cow, but from the average nightly catch with man the total for four nights with man has been calculated (de Zulueta's text shows that the calf and donkey figures in his table have been transposed). Similarly, Sasa & Sabin (1950) made 13 trials with man and 15 with goat; but from the nightly average the total catch with goat for 13 nights has been calculated.

The ratios obtained in Table VI place *A. funestus* Giles and *A. gambiae* Giles as the most anthropophilous species, and this agrees with their general reputation. Haddow (1942) in Kenya used two identical huts 30 yd. apart, one baited with a man, the other with a calf; Smith (1955) in Tanganyika used one hut containing two adult humans and a child, three cows and three goats, plus three men catching the mosquitos. It is rather surprising to find *A. funestus* much more anthropophilous than *A. gambiae*, which is the more important malaria vector, but a third investigator in Kenya (Garnham, 1938) also found *A. funestus* more attracted to man than *A. gambiae*, and recently Smith & Draper (1959) have demonstrated the same thing on the Kenya-Tanganyika border. The considerably greater longevity of *A. gambiae* (Davidson, 1955) probably explains its greater importance as a malaria vector.

De Zulueta (1952) in Colombia used two stable traps 100 m. apart with a man in one and a calf in the other. He found a great contrast between the malaria vector *A. darlingi* Root, which was more attracted to the man, and the other Anophelines, mainly *A. pessoai* Galvão & Lane, which were much more attracted to the calf. Haddow found the same contrast between the vectors, *A. gambiae* and *A. funestus*, and the non-vector, *A. coustani* Lav.

Sasse & Hackett (1950) in Peru used a single stable trap, changing the bait each night. They found that *A. pseudopunctipennis* Theo. was attracted in rather larger numbers to a calf than to a man.

Senior White (1952) in Trinidad used dawn traps (in principle the same as window-trap huts) placed about 30 yd. apart, and found cow much more attractive than man to *A. aquasalis* Curry.

This list of Anophelines roughly spans the range of the world's regular malaria vectors from the most efficient (*A. gambiae* and *A. funestus*) to one of the least efficient (*A. aquasalis*). The point to note is that the order in which they have been placed by their attraction ratios is also more or less the order of their importance as malaria vectors. The range of their attraction ratios is from an average of about 38:1 in favour of man for *A. funestus* to 1:11 in favour of cow for *A. aquasalis*; the Malayan vectors fall within this range (see p. 53).

Sasa & Sabin (1950), investigating Culicines in Japan, used ordinary bed mosquito nets slightly raised on one side and enclosing the bait; they collected the mosquitos in the morning. With the two commonest species their results produced ratios quite similar to those for the equivalent species in Malaya. They found *C. pipiens pallens* Coq. was more attracted to man than to goat (ratio 3.8:1 compared with 2.8:1 for Malayan *C. p. fatigans*), whilst *C. tritaeniorhynchus* was more attracted to goat (ratio 1:2.3 compared with 1:1.5 for Malayan *C. t. summorosus*). With *C. vishnui*, which was more attracted to goat, the man:goat ratio, 1:5, is somewhat different from the Malayan result (1:1.2) with the related species *C. 'annulus'*, but the numbers of *vishnui* are rather small and it is a different species.

The effect of the type of trap.

Certain species that were captured by Wharton (1951a, b) in his experiments with window-trap huts were also caught in the net traps, so that it is possible to compare the effect of the two types of trap on the attraction ratios (Table VII).

As mentioned on p. 51, the important point shown by Table VII is that although the values of the attraction ratios obtained with the two types of trap differ considerably, the order in which the species are placed by these ratios is the same. With regard to the differing values of the ratios, the window-trap hut seems to magnify whatever bias is displayed in the net trap. Thus, the man:calf ratio for the anthropophilous species *C. p. fatigans*, which was 1.7:1 in the net trap, increased to 4:1 in the window-trap hut, while with the other species, which

were all zoophilous, the ratios were increased in favour of the calf by roughly tenfold.

Why the window-trap hut should have magnified the bias is not clear, but one reason might be its relatively narrow louvred entrances. Presumably these formed an obstacle preventing more than a few mosquitos entering, unless the bait inside was particularly attractive. This would be likely to apply especially to all the species in Table VII, except *C. p. fatigans*, as they bite more readily out of doors (Wharton, 1951a, b).

TABLE VII.

Comparison of the man:calf attraction ratios obtained with net traps and with window-trap huts. Figures for window-trap huts from Wharton (1951a, b).

Species	Net traps			Window-trap huts		
	2 men	1 calf	Ratio men : calf	1 or 2 men	1 calf	Ratio men : calf
<i>Culex pipiens fatigans</i> ..	909	531	1.7 : 1	442	111	4.0 : 1
<i>Culex 'annulus'</i> ..	166	353	1 : 2.1	48	942	1 : 20
<i>Mansonia uniformis</i> ..	243	687	1 : 2.8	16	582	1 : 36
<i>Anopheles indiensis</i> * ..	6	36	1 : 6.0	0	61	1 : > 61
<i>Culex gelidus</i> ..	688	5,328	1 : 7.8	25	2,658	1 : 106
<i>Anopheles vagus</i> ..	12	985	1 : 82	0	122	1 : > 122

* This species belongs to the *hyrcanus* group and is referred to as *A. hyrcanus* by Wharton (1951a), but the material was in fact mainly of *A. indiensis* (see Wharton, 1953); the figures for the net trap are from Table III in the present paper.

Percentage of mosquitos blood-fed.

The figures for the number of mosquitos that were found to be blood-fed, presumably from biting the bait, have only a limited value because the human bait was more or less protected by bed nets and clothing, so that the figures for human bait are not comparable with those from the calf and goat baits. However, there are a few points of interest in Table VIII.

It will be seen that with both Anophelines and Culicines (except *C. p. fatigans*) from the goat-baited nets, the percentage of those examined which had taken blood was only about half that from the calf-baited nets; for all mosquitos the figures were 29 per cent. (goats) compared with 59 per cent. (calf). This supports the evidence from the total numbers caught, which showed that a calf was considerably more attractive than two goats.

The figures from the man-baited nets show considerable differences between species in the percentage blood-fed, possibly because the bed nets deterred some species more than others. For example the percentage of *A. letifer*, *A. sundaicus* and *C. p. fatigans* with blood was very low (under 10 per cent.), but in window-trap huts without insecticide, where the human bait was not protected by nets, Reid & Wharton (1956) found that these species engorged freely (75 to 95 per cent. blood-fed). On the other hand there were species, such as the dark-winged form of *A. barbirostris*, *Aë. 'butleri'*, *C. 'fraudatrix'*, and *M. annulata* and *M. 'dives'*, of which a substantial percentage (23-39 per cent.) were blood-fed

despite the bed nets. It is possible that these are rather bold, quick-biting species which were often able to obtain a blood-meal in the periods when the men were not beneath their bed nets.

TABLE VIII.

The percentage of mosquitos that took blood.*

Species	Percentage blood-fed from traps baited with		
	2 men	1 calf	2 goats
<i>Anopheles</i>			
<i>baezai</i>	14	62	41
<i>barbirostris</i> (dark-winged)	23	66	26
<i>barbirostris</i> (light-winged)	7	47	46
<i>kochi</i>	0	23	16
<i>lesteri</i>	15	90	34
<i>letifer</i>	3	64	—
<i>separatus</i>	17	82	35
<i>sundaicus</i>	8	34	19
<i>tessellatus</i>	5	66	17
<i>umbrosus</i>	20	63	—
<i>vagus</i>	0	78	47
Total Anophelines	16	69	32
<i>Aedes</i>			
'butleri'	39	82	50
<i>Culex</i>			
<i>pipiens fatigans</i>	3	7	21
'fragilis'	4	3	0
'fraudatrix'	28	87	6
<i>fuscocephalus</i>	0	15	18
<i>gelidus</i>	6	60	10
<i>tritaeniorhynchus</i>	4	19	13
'annulus'	7	54	23
<i>Mansonia</i>			
<i>annulata</i>	37	70	—
'dives'	32	71	55
<i>uniformis</i>	10	74	56
Total Culicines	15	53	26
Grand Total	15	59	29

* For the Anophelines, the figures on which these percentages are based are the total caught as shown in Table IV, combining the results from the two columns for man-baited traps. For the Culicines, the figures are a little less, as not all those caught were examined for blood. Only species of which 50 or more individuals were examined from at least one bait are included.

Discussion.

Bates (1949), in discussing the host preferences of mosquitos, reviews some of the results of precipitin tests and points out the difficulty of comparing the blood preferences of different species. He says (p. 75) "it would in particular, be valuable to have an experimental method whereby species in different parts of the world could be compared with regard to host-seeking habits". It seems, as Table VI shows, that the results of comparative trapping, if expressed as attraction ratios, provide such a method.

The great value of the precipitin test is that it can reveal the actual blood-feeding pattern of mosquitos at a particular time and place. However, since this pattern is formed by a combination of the availability of suitable hosts and the host preferences of the mosquitos, it varies from place to place and time to time as the number of suitable hosts varies. Consequently the precipitin test is not very satisfactory for comparing the preferences of mosquitos at different places and times. With comparative trapping, a fixed number of hosts is offered under more or less standardised conditions, so that preferences revealed by the attraction ratios at different places and times can be compared. It should be relatively easy by comparative trapping to discover if changes occur in the host preferences of a mosquito species due to such factors as seasonal changes or the application of residual insecticides.

A little should be said about the methods to be employed in comparative trapping. The essentials are that in any particular trial the baits being compared must be exposed at the same time and place, in the same type of trap, and for the same length of time. Otherwise, differences in the number of mosquitos caught with each bait cannot be attributed solely to differences in the attractiveness of the baits. If the position of the baits can be alternated, so much the better.

What type of trap and what species and size of bait animals to employ are largely matters of choice governed by local circumstances. It is too early yet to try to standardise the trap and the bait; the important thing is for investigators to state clearly what methods they use. Where the object is to investigate vectors of human disease, the man/cow comparison has much to recommend it. Only the more anthropophilous species will be attracted in larger numbers to man, and there is already a considerable body of data comparing man with cow (or calf). Horses, donkeys and pigs are probably suitable alternatives to cow. Each type of trap or catching method has advantages and disadvantages, but stable traps (Magoon, 1935) would seem to avoid the main drawbacks of net traps.

There remains one point of importance to discuss; the distance between the traps. It appears that they should be far enough apart not to interfere with one another, otherwise, as happened at site 2 where there were goats within 5-10 yd. of the animal-baited trap (p. 48), the more favoured bait attracts an unduly large number of mosquitos, and the attraction ratio is distorted. A suitable distance is probably about 50 yd.

This leads to an important theoretical conclusion. If in fact there was no interference between the baits when they were 20-50 yd. apart, presumably mosquitos were not subjected simultaneously to stimuli from both baits, and the experiments have been comparing the power of each bait, independently of the other, to attract mosquitos. This was certainly so in the experiment of Sasse & Hackett (1950) with *A. pseudopunctipennis*, because the baits were exposed one at a time on alternate nights (see p. 56). This explains why the word preference has been avoided as much as possible in this paper and attraction used instead; preference may suggest a choice between alternatives (the two different baits), and this does not seem to have occurred. To pursue this subject any further would take us into the difficult and still largely speculative question of how wild mosquitos find their hosts, and space does not permit.

Summary.

Experiments were made at three different sites in Malaya between 1952 and 1957 to compare the numbers of mosquitos attracted to man, calf or goat under the same conditions. The baits being compared (either two men and one calf or two men and two goats) were exposed simultaneously in two net traps placed about 50 yd. apart. This procedure is referred to as comparative trapping.

The total number of mosquitos caught with each bait showed calf to be much

the most attractive, followed by goats and then men. However, there were three species, the dark-winged form of *Anopheles barbirostris* Wulp, *Aedes albopictus* (Skuse) and *Culex pipiens fatigans* Wied. that were attracted in larger numbers to man than to calf, and six species attracted in larger numbers to man than to goat.

To compare the host preferences of the different species as between man and calf, the numbers of each caught with man and with calf have been expressed as a ratio (the man:calf attraction ratio). For most species the ratios from the different sites were much the same, showing that the method produced repeatable results. The data from the three sites were therefore combined.

On the combined data the ratios range from 3.4:1 in favour of man for the dark-winged form of *Anopheles barbirostris* to 1:82 in favour of calf for *A. vagus* Dön.

As might be expected, the ratios show that the vectors of malaria, filariasis and virus diseases are among the more anthropophilous species.

Attraction ratios have been calculated from suitable data published by various authors for ten different species in five countries. The ratios obtained place the Anophelines in an order that agrees well with their status as vectors of malaria.

It appears that if the results of comparative trapping are expressed as attraction ratios, this offers a method of comparing the host preferences of mosquitos of the same or different species, at different times and places in a country, or even in different countries.

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OBSERVATIONS ON THE EFFECTS OF CAROTENE ON THE GROWTH AND PIGMENTATION OF LOCUSTS.

By R. H. DADD¹

E. M. N.

Department of Zoology and Applied Entomology, Imperial College of Science and Technology.

Introduction.

In developing artificial diets for locusts it was found that the pink and yellow colours characteristic of gregarious hoppers of *Schistocerca gregaria* (Forsk.) reared in the normal way on grass were absent in those reared on artificial diets devoid of carotene. With carotene in the diet, hoppers developed the colours in question, although rarely with the normal intensity. At first it appeared that growth might be adversely affected by the absence of carotene, but later experiments with an improved yeast-free basal diet showed that, while pigmentation was affected as before, carotene was not required for growth and development (Dadd, 1957, 1960).

Locusta migratoria (L.) could be reared on the improved basal diet and was also not dependent on carotene for growth. No changes in pigmentation obviously related to the presence or absence of carotene were noted in this species, but this was scarcely surprising as carotenoid pigments contribute little to the superficial coloration of *Locusta* hoppers (Goodwin, 1952). It was, however, noteworthy that all synthetic diets tended to induce unusual coloration. Gregarious *Locusta* hoppers are bright orange below and velvety black above. On synthetic diets the dorsal surface frequently became more or less velvety black, but in no case has the ventral surface been orange. By far the majority of hoppers have been a sooty grey or mottled brown colour dorsally and a dirty pale brown ventrally, corresponding to the colour types B-F of Gunn & Hunter-Jones (1952) which tended to arise under conditions of low-density rearing.

As experiments with the improved diets showed beyond doubt that dietary carotene was not required for growth to the adult stage, it was difficult to account for the apparent tendency for growth to be improved by carotene in the early experiments with *Schistocerca*. A hypothesis put forward to account for this situation (Dadd, 1960) rested on the assumption that, supposing carotene to influence growth, the carotene present in the normal egg might be enough for good growth under otherwise satisfactory dietary conditions, but might be inadequate with the very inferior nutritional conditions provided by the early yeast-containing diets. On this view the great variability of the early experiments could be interpreted as a reflection of differing amounts of carotene initially present in different groups of eggs. Were carotene present in the egg in low concentration, dietary carotene might affect growth markedly; if in high concentration in the egg, its presence in the diet would have little effect.

Had it been possible to obtain viable offspring from locusts reared on carotene-free synthetic diets this hypothesis could have been checked experimentally, for eggs produced under these circumstances could contain only traces of carotene. Unfortunately no locusts reared on synthetic diets matured properly or reproduced, although on several occasions adults were kept for periods of three to four months to give them the opportunity of doing so. However, when artificial food mixtures of bran, dried milk, yeast and grass meal were shown to

¹ Now at Entomology Research Institute, Belleville, Ontario.

give growth comparable to that obtained with fresh grass, the production of eggs which would probably contain little carotene became feasible.

The composition of a diet of this sort (hereafter termed the Howden diet) which supported satisfactory nymphal growth in *Schistocerca* is given by Howden & Hunter-Jones (1958). They noted that unless a small proportion of fresh grass was included, pigmentation was impaired and no viable eggs were produced. In their experiments the coloration of nymphs reared without grass supplementation resembled that obtained with carotene-free synthetic diets (author's observation) and for this reason it seemed likely that the Howden diet was deficient in carotene, and any eggs produced could be expected to be similarly deficient. Unfortunately such eggs were not viable, and the grass needed to ensure viability also allowed normal coloration to develop and thus made it uncertain that carotene was in short supply. Even so, with the small amount of supplementary grass used it was likely that eggs having minimal amounts of carotene would be obtained, and it would have been worth examining the effect of dietary carotene on hoppers from such eggs.

In the event, no such eggs were forthcoming from *Schistocerca* while this work was in progress, but *Locusta* material was available. The original Howden diet was unsatisfactory for *Locusta* (Howden & Hunter-Jones, 1958), but a modification having only one-tenth the amount of yeast was found to allow improved growth and reproduction (this change, incidentally, rendered the diet unsatisfactory for *Schistocerca*). Offspring from parents thus reared were therefore used to test the effect of dietary carotene on what, it was hoped, were carotene-deficient hatchlings. During the course of these experiments, supplies of grass meal used in compounding the parental diet ran out and a further modification was introduced; this consisted in replacing both grass meal and the small supplement of fresh grass by an approximately equivalent amount of grass that had been dried in the laboratory a few days before use. As hatchlings derived from parents thus fed gave results which were similar to those from parents given the earlier diet, no distinction is made between these two sources of hatchlings in the following account.

It will be convenient henceforward to refer to eggs or hatchlings derived from parents reared on Howden-type diets as 'modified eggs or hatchlings'. The use of such modified hatchlings unmasked a profound effect of dietary carotene in *Locusta*, and a further attempt was made to procure similarly modified hatchlings of *Schistocerca* from parents reared on the dried-grass diet. Unfortunately only one of these specially reared adults survived to oviposit, and of the three pods it laid, only one hatched viable hoppers. Although this one group of hatchlings gave results which were similar to those obtained with *Locusta*, conclusions based on them must necessarily remain tentative in the absence of confirmatory experiments.

The account which follows is mainly concerned with the results of growth trials with modified hatchlings using two diets differing only by the presence in one of carotene. The most noteworthy effect of dietary carotene concerned pigmentation, and, for reasons which will become apparent, prompted an examination of the blood pigments of *Schistocerca* hoppers.

Materials and methods.

Schistocerca and *Locusta* are kept in gregarious stock culture at the Anti-Locust Research Centre, London, and, when required, pods of normal eggs ready to hatch were usually available. The staff of the Centre very kindly co-operated in rearing parental locusts on the special diets already discussed; any egg-pods laid by these insects were incubated at the Centre until they hatched, and were then collected and used in growth experiments within 24 hours.

The procedures followed and the preparation of synthetic diets have been described elsewhere (Dadd, 1960). The composition of the basic synthetic diet used in the present experiments is given in Table I. Growth data were obtained

TABLE I.

Composition of the basic synthetic diet.

Cholesterol	50 mg.
Linoleic acid (B.D.H. technical grade)	0.2 ml.
β -carotene (Light & Co. synthetic)	25 mg.
Salt mixture (Glaxo DL.6)	1.5 g.
Sucrose	5.0 g.
White dextrin (Hopkins and Williams)	5.0 g.
Cellulose powder (Light & Co.)	15.0 g.
Casein (B.D.H. fat- and vitamin-free)	6.0 g.
Bacteriological peptone (B.D.H.)	2.0 g.
Egg albumen powder (B.D.H.)	2.0 g.
Ascorbic acid	100 mg.
Vitamins in 10 ml. of 20% ethanol:	
thiamin	25 μ g./g. of diet
riboflavin	25 " "
nicotinic acid	100 " "
pyridoxine	25 " "
calcium pantothenate	50 " "
meso-inositol	250 " "
folic acid	25 " "
p-aminobenzoic acid	25 " "
biotin	1 " "
choline chloride	1250 " "

by weighing hoppers individually every two days when the rearing jars were cleaned out and fresh diet provided. From the 18th day of an experiment all hoppers were sexed on being weighed. From time to time detailed notes were made on the colour of hoppers, and on their relative activity; when adult, biometric data were recorded for all that emerged perfectly.

Observations on the colour of *Schistocerca* haemolymph derive mainly from records kept on several hundred fourth- and fifth-instar hoppers used in work on the concentration of ascorbic acid in the blood. This information, which covered hoppers reared on grass as well as on synthetic diets, was subsequently checked from time to time on any hoppers that happened to be available from other experiments. With the kind co-operation of Dr. C. A. Wright and Mr. G. C. Ross of the British Museum (Natural History) it was possible to separate and compare the pigments present in different colour types of *Schistocerca* blood by means of strip electrophoresis. The apparatus used has been described by Ross (1959). Untreated whole blood was run for about two hours in barbitone buffer of pH 10.4 on cellulose acetate strips 2.5 cm. wide with a current of 1 mA per strip. The movement of the pigments could be observed visually, and the position of the various protein fractions that separated was shown subsequently by staining with either ponceau S or nigrosin. It was thus possible to relate the visible pigments to particular protein fractions.

Results.

The effect of dietary carotene on modified hatchlings.

In this set of experiments, seven groups of modified *Locusta* hatchlings were each divided and reared on two synthetic diets differing only by the presence in one of carotene. Some groups were the issue of one pod, but where two or more pods hatched together the mixed hatchlings were used as a group. In the first

two experiments groups of hatchlings from normal eggs (*i.e.*, eggs laid by parents reared on grass) were similarly split and reared on the two diets. The third experiment included a group from a pod laid by a female which, although reared to maturity on the Howden diet, had been allowed fresh grass for a day after a long failure to oviposit; shortly after this meal it produced the pod in question. Growth data from these experiments are summarised in Table II.

TABLE II.

Growth data for groups of normal and modified *Locusta* hatchlings reared on a synthetic diet with and without carotene.

Exp.	Type of hopper	Treatment	Mean weight (mg.) of groups of hoppers and numbers alive (in brackets) at these times after hatching				Adults obtained		
			6 days	12 days	22 days	32 days	♂♂	♀♀	aborts
1	modified	no carotene	57 (19)	173 (11)	744 (4)	1001 (3)	1	3	
	„	carotene	64 (20)	182 (17)	730 (11)	1110 (6)	5	4	1
	normal	no carotene	63 (20)	199 (17)	851 (12)	1228 (10)	3	8	
	„	carotene	65 (20)	192 (13)	907 (9)	1254 (8)	5	4	
2	modified	no carotene	26 (2)	114 (1)	all dead	—	0	0	
	„	carotene	37 (6)	144 (2)	618 (1)	900 (1)	1	0	
	normal	no carotene	53 (11)	161 (10)	496 (8)	920 (7)	3	4	1
	„	carotene	52 (20)	150 (17)	484 (14)	970 (8)	7	4	
3	modified	no carotene	50 (22)	93 (11)	344 (7)	741 (6)	2	3	
	„	carotene	55 (21)	148 (18)	415 (16)	928 (15)	9	6	
	modified + 1 day grass }	no carotene	57 (14)	150 (11)	454 (6)	956 (4)	4	1	
		carotene	68 (10)	182 (9)	519 (5)	943 (2)	3	1	
4	modified	no carotene	45 (17)	122 (14)	365 (9)	876 (5)	3	3	
	„	carotene	48 (20)	117 (14)	405 (10)	932 (6)	4	3	
5	modified	no carotene	58 (16)	157 (13)	519 (11)	905 (8)	6	3	
	„	carotene	56 (17)	141 (15)	615 (10)	979 (4)	6	1	
6	modified	no carotene	31 (20)	70 (12)	289 (6)	490 (2)	1	0	
	„	carotene	40 (5)	118 (5)	399 (3)	922 (3)	1	2	
7	modified	no carotene	58 (12)	148 (12)	524 (9)	940 (7)	4	1	
	„	carotene	60 (18)	151 (15)	605 (9)	1189 (5)	2	4	1

Dietary carotene is clearly not essential for growth to the adult stage of modified *Locusta* hatchlings. However, all experiments show a tendency for the mean weights of groups given the diet lacking carotene to become less than those of corresponding groups given carotene, so that the maximum nymphal weights and final adult weights were lower. This growth difference, apparent in the data of Table II where no account is taken of sex, was clarified by separating the pooled results of all experiments with modified hoppers according to sex and plotting graphs to show the growth of each sex on the two diets (fig. 1). Without carotene the growth of both sexes was evidently retarded from the third instar (about the 12th day) and although not apparent from the shape of the mean growth curves, the final moult was on the whole delayed and spread over a longer period. When adult growth had finished, mean weights of males and females on the two diets differed by about 150 mg. and 300 mg., respectively. Biometric data indicated that the linear dimensions of adults reared on the diet without carotene were slightly smaller (Table III).

The most striking effects of the difference in diet concerned pigmentation. It should be mentioned that modified hatchlings were always initially pale buff in colour, sometimes with light brown stripes on the buff ground. Normal *Locusta*

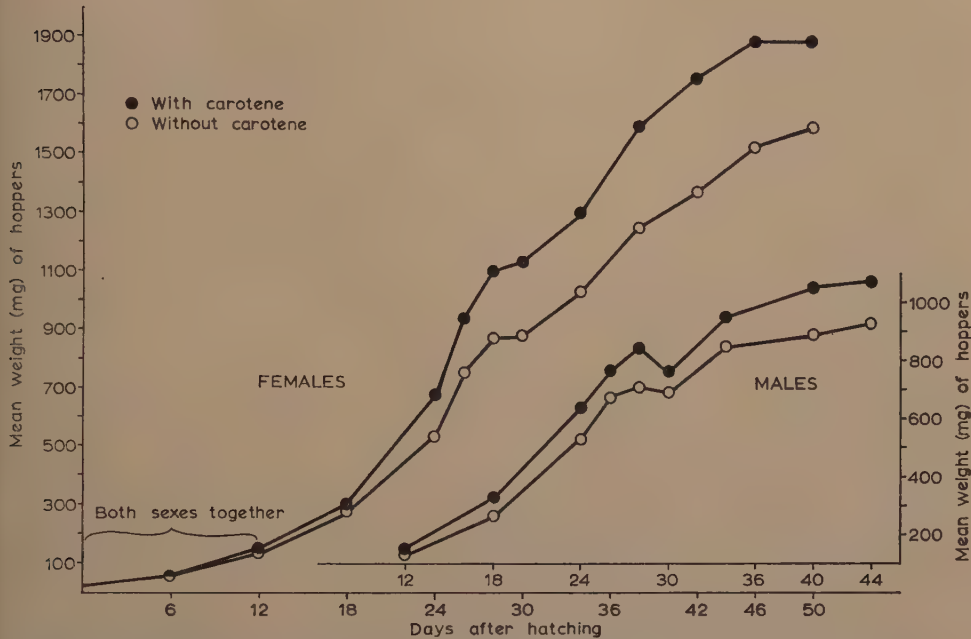


Fig. 1.—Mean growth curves for modified *Locusta* hatchlings reared on a synthetic diet with and without carotene; curves based on the pooled data of all experiments after separation by sex.

hatchlings are mostly dark, although an occasional pod gives pale buff-coloured hoppers and, more frequently, hoppers having dark stripes on a buff ground. After the first moult a consistent difference in colour was observed between

TABLE III.

Morphometric data for adults obtained in growth experiments in which normal and modified *Locusta* hatchlings were reared on a synthetic diet with and without carotene.

Type of locust and diet		Means values (Elytron, femur and caput in mm.)					No. of individuals measured	
		E	F	C	E/F	F/C		
Modified hatchlings : males	no carotene	37.2	20.4	5.9	1.82	3.44	15	
		38.4	20.9	6.1	1.84	3.45	22	
	females	no carotene	43.2	23.3	7.1	1.86	3.25	6
			45.0	24.3	7.3	1.87	3.32	14
Normal hatchlings : males	no carotene	37.3	20.1	6.4	1.87	3.15	3	
		37.6	20.8	6.2	1.81	3.37	8	
	females	no carotene	42.5	22.9	7.3	1.86	3.16	6
			45.5	24.4	7.4	1.87	3.31	9

hoppers on the two diets. All those lacking dietary carotene remained buff-coloured or a variegated brown, whereas those given carotene showed some degree of melanisation, in most cases with areas of velvety black dorsally. This difference increased with subsequent moults until, by the fourth instar, hoppers given carotene were either sooty black and grey all over, or brown ventrally and velvety black dorsally; all those without carotene remained buff or mottled brown, or in the fourth and fifth instar developed a pale greenish-blue colour, particularly on the head and pronotum. On becoming adult those given carotene had, without exception, purplish and black markings typical of gregarious adults; those without carotene were either pale brown or with greenish-blue areas, the latter type constituting about 30 per cent. of the total.

These differences in pigmentation were essentially similar to those which distinguish the solitary and gregarious phases of *Locusta*, except that a bluish colour occurred rather than the grass-green of true solitaries. Moreover, the activity of the two sets of hoppers differed. It is difficult to be precise about this in the absence of quantitative data, but during routine weighing it was strikingly obvious that hoppers on the carotene-containing diet were more active when disturbed and jumped with far greater force. Numbers, particularly during the early instars before mortality was heavy (see Table II), were such as to exclude rearing density as a direct cause of these differences. In this connection it may be noted that in experiment 2 (Table II) where only one modified hopper survived beyond the third instar on the carotene-containing diet, its colour remained typically 'gregaroid' throughout subsequent development to the adult stage, although on grounds of density it might have been expected to develop *solitaria* coloration after one moult (Gunn & Hunter-Jones, 1952).

In contrast to pigmentation and activity, the shape of all hoppers, regardless of diet, appeared superficially (as judged particularly by the shape of the pronotum) to be gregaroid rather than solitaroid. However, the morphometric data given in Table III, while indicating no difference between modified hoppers given the two diets, do indicate that *all* hoppers had, if anything, slightly solitaroid morphometric ratios, if comparison is made with the laboratory and field data tabulated by Gunn & Hunter-Jones.

In contrast to the extreme differences in pigmentation obtained with modified hoppers, normal hoppers melanised strongly whether dietary carotene was present or not. In experiment 1 (Table II), hatchlings were of the initially black type and no colour differences whatever were discernible. The hatchlings used in experiment 2 were exceptionally small (with an average weight of 14 mg.) and of the unusual uniformly buff type. In this group a slight difference in the degree of melanisation was detected, but it would have passed unnoticed if not specifically looked for. Hatchlings from the parent allowed fresh grass for one day all became melanised regardless of diet, although again a slight difference in the degree of blackening could be detected. This result was of particular interest in indicating that very small amounts of grass in the parental régime were sufficient to mask subsequent dietary differences in carotene. It will be recalled that a very small amount of fresh grass added to the original Howden diet was sufficient to ensure normal pigmentation in *Schistocerca* hoppers.

Of the three pods of modified eggs of *Schistocerca* only one gave rise to viable hatchlings, about 20 in number. All were totally unmelanised and had an average weight of 15 mg. (the average weight of hoppers from normal pods is usually in the range 18-25 mg.). They were a very pale brown in colour, quite unlike the greenish unmelanised hatchlings which from time to time form a proportion of the issue of normal egg-pods. The two other pods gave rise to hatchlings which had normal black markings, but were very small (average weights 12 mg. and 16 mg.) and feeble, and mostly died within 24 hours of hatching.

The group of viable hatchlings was split between the two diets (7 per diet)

and their subsequent growth is represented graphically in fig. 2. By the sixth day, half of them had died on both diets and it was therefore possible to follow the growth of the remainder individually. It will be seen that although none of the hoppers given carotene survived to become adult, they all grew at a greater rate than those lacking carotene. Three hoppers on the diet without carotene attained the final moult, when one aborted and the other two emerged but, failing to expand and harden, died.

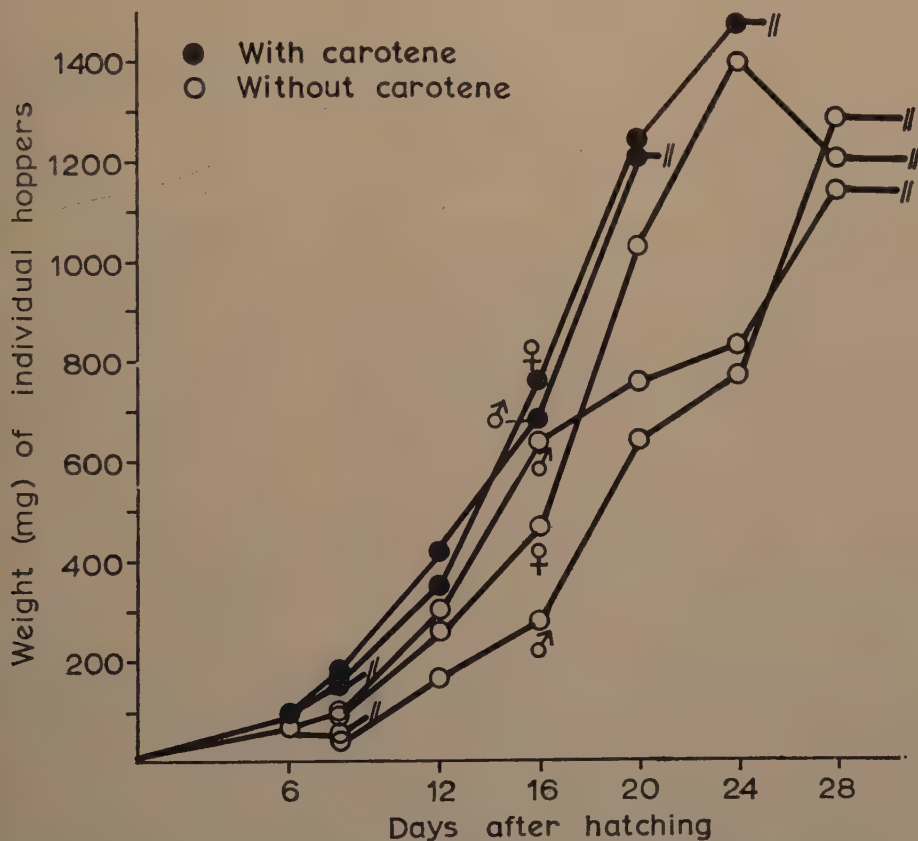


Fig. 2.—Growth curves for individual modified *Schistocerca* hatchlings reared on a synthetic diet with and without carotene. Vertical lines on curve indicate death of hopper.

Some pigmentation effects analogous to those obtained with *Locusta* were observed in this experiment. On the carotene-deficient diet, the four second-instar hoppers surviving on the sixth day showed only faint traces of melanin, and, as has always been the case with *Schistocerca* hoppers reared without carotene, no pink coloration. One of the three survivors on the diet containing carotene was fully melanised and had a strong pink tinge; the other two were slightly less pigmented. In the third instar these three hoppers became fully melanised and all had a strong pink tinge, whereas those on the diet lacking carotene were still largely unpigmented and unmelanised. These latter developed more black pigmentation in the fourth instar, but even so remained unusually pale throughout later development.

The pigmentation of the haemolymph of Schistocerca.

The most striking features of the experiments with modified *Locusta* hatchlings was the apparent tendency for solitaroid coloration to develop under gregarious rearing conditions in the absence of dietary carotene. In particular, the unusual bluish colour which appeared in about 30 per cent. of the adults required consideration, for although the author has never seen a locust of phase *solitaria*, they are always described as grass-green in the extreme condition, never blue-green. Now solitaries owe their green colour to an integumental pigment of the insectoverdin type which is known to be a complex chromoprotein containing a blue prosthetic substance, probably mesobiliverdin, and a yellow carotenoid prosthetic complex of β -carotene and astaxanthin (Goodwin & Srisukh, 1951). If, as seemed clearly the case, the absence of dietary carotene induced a tendency for insectoverdin to develop, it will be apparent that with no β -carotene available for the yellow carotenoid prosthetic complex (astaxanthin is formed from β -carotene and would thus be absent as well), only the blue prosthetic substance, mesobiliverdin, could appear in the pigment formed. As we have seen, bluish locusts were produced.

These considerations brought to mind an occasion when this same greenish-blue colour had been noted in *Schistocerca*. While making ascorbic acid determinations on the blood of several hundred nymphs of this species, it was noticed that whereas grass-fed gregarious nymphs had, with few exceptions, brilliant yellow blood, nymphs reared on synthetic diets lacking carotene had turquoise-blue blood in many cases. From notes kept on each individual used it was found that 264 out of 280 hoppers reared on grass had yellow blood (the remainder had greenish blood) and 52 out of 56 fifth-instar hoppers reared on synthetic diet had blue blood (in the remainder it was colourless). The proportion of fourth-instar hoppers having blue blood was much less (19 out of 54), suggesting that the blue pigmentation develops mainly during the last instar. This blue blood could only be accounted for by supposing that, given diets without carotene, hoppers had a tendency to produce insectoverdin, which, in the absence of the yellow prosthetic substance, β -carotene, resulted in the production of a blue chromoprotein containing mesobiliverdin.

On the hypothesis that the pigments of yellow and blue blood might be chromoproteins identical with those constituting the insectoverdin of green blood, it seemed worth attempting a comparative separation by electrophoresis. Yellow and blue bloods were obtained from normal gregarious fifth-instar hoppers, and hoppers reared on carotene-free synthetic diets, respectively; as *solitaria* hoppers were not available, green blood from fifth-instar albino hoppers was used. Good separation of the pigments occurred, and tracings of the bands obtained from drops of blood run in parallel are shown in fig. 3. Duplicate spots of each type of blood were run in parallel and one of each pair was subsequently stained to show the protein fractions present. The protein bands shown in the figure were stained on the same strips from which the pigment bands were drawn, after these had been outlined in pencil.

Migration of the pigments could be followed visually. Green blood gave rise to a yellow and a blue band advancing towards the positive pole, the yellow band moving most rapidly. Blue blood gave a blue band which moved parallel to the blue band of the green blood. Yellow blood gave a yellow band which moved parallel to the yellow band of green blood. On staining the duplicate papers for protein, five well-defined bands were found to have separated from each blood. Some of these showed signs of further subdivision, and in more critical conditions might be shown to be complex; moreover, subsequent work showed that at least two protein fractions migrated in the opposite direction. It is not claimed, therefore, that the five fractions here discussed represent the complete number of distinct blood proteins. Numbering these protein bands from the origin (see

fig. 3), in all cases the blue pigment coincided with band 3 and the yellow pigment with band 5. Band 1 migrated scarcely at all, and was associated with a dirty brownish coloration, most noticeable in the case of green blood, and probably due to melanic oxidation products.

From these results it is fairly clear that the blue pigment found in the blue blood of hoppers reared on carotene-free synthetic diets is identical with the blue component of insectoverdin. As the same five protein fractions appear in each type of blood whether prosthetic pigments are present or absent, it may be argued that the β -carotene and mesobiliverdin of insectoverdin are combined with different

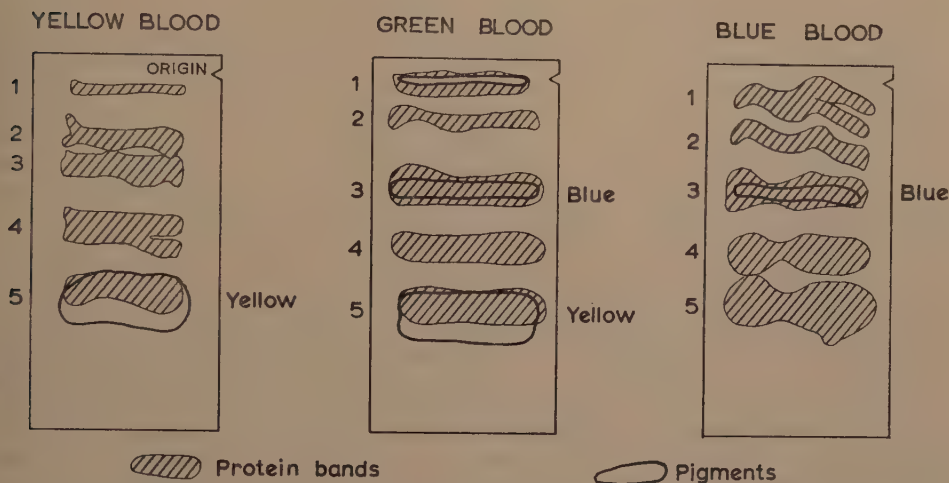


Fig. 3.—Diagrams of the protein and pigment bands which separated on electrophoresis of yellow, green and blue blood from fifth-instar nymphs of *Schistocerca*. For further explanation, see text.

fractions of the blood proteins, for were the same blood protein involved with both pigments, so that the differential mobilities of bands 3 and 5 were those of the two chromoproteins as a whole rather than of their protein components alone, one would expect to find a protein band missing from the positions corresponding to the absent pigments in the blue and yellow bloods, and this was not the case.

It may be remarked that on examination of the strips under an ultra-violet lamp an intense yellow-green fluorescence occurred in the region of the yellow pigment and a faint red fluorescence in the middle of an absorptive band corresponding to the blue pigment. The yellow fluorescence consisted of two contiguous but sharply distinct bands of slightly different colour and perhaps indicated that in drying out the strips some breakdown of β -carotene from its protein complex occurred. The significance of the red fluorescence is unknown.

Miscellaneous observations on the pigmentation of locusts reared on synthetic diets.

Carotene was first thought to be of nutritional importance to locusts when it was observed that, without it, hoppers of *Schistocerca* failed to develop a pink tinge in the early instars (caused by free insectorubin) or a vivid yellow ground colour in the later instars (caused by integumental carotenoids). In place of the vivid yellow, areas of a faint yellow grey and green occurred. These were presumably due to the small amounts of carotene present in the egg, the greenish

component indicating additionally a slight amount of integumentary mesobiliverdin, for, as we have seen, this pigment tends to develop in the absence of carotene. Further, the experiments described in this paper implicate carotene in the processes which govern melanisation.

Although dietary carotene to some extent ameliorated these pigmentation abnormalities, it was early noted and subsequently confirmed throughout these studies that even with amounts of β -carotene considerably in excess of those normally found in green plant tissues, pigmentation, particularly the yellow colour due directly to integumental carotene, rarely developed with the intensity found in nymphs reared on grass. This suggested some failure in the uptake or utilisation of carotene when supplied in synthetic diets, and internal examination of hoppers so reared strikingly supported this view.

The blood of grass-fed gregarious hoppers is a vivid yellow in colour, due to a chromoprotein containing β -carotene, and, as we have seen, that of gregarious *Schistocerca* hoppers reared on carotene-free synthetic diets is either blue or colourless. With carotene in the diet the blood may be colourless, blue, slightly greenish-blue, pale green, or a very faint yellow, but never a strong green or yellow. Quite clearly, no more than traces of carotene ever occurred in the blood of hoppers reared on synthetic diets. In only a few cases was the blood of *Locusta* hoppers examined, and in these it was always colourless, whether the diet contained carotene or not.

The clearest indication that artificial diets failed to provide suitable conditions for the uptake of the carotene they contained was given by the appearance of the fat-body. In both *Schistocerca* and *Locusta* this organ is always coloured yellow in nymphs reared on grass by the β -carotene it contains. On synthetic diets, whether they contained carotene or not, the fat-body was always found to be white. It must therefore be concluded that although some of the carotene provided in synthetic diets must have been absorbed (otherwise carotene-dependent effects could not have been demonstrated experimentally), the amounts must always have been grossly sub-optimal.

Discussion.

In nutritional studies it is sometimes the case that individual insects of the same species respond to particular dietary deficiencies with very different rates of growth; to account for this the suggestion has been made that if growth factors omitted from the diet were present in variable amount in the egg, requirements for them during subsequent growth might be masked in varying degree. Similarly, the inability of most insects reared on synthetic diets to reproduce successfully has been attributed to the presence of ovarian supplies of unrecognised essential factors in amounts sufficient for larval growth but inadequate for egg-production.

The use of modified *Locusta* hatchlings afforded a clear demonstration that β -carotene was involved in a situation of this sort. Such hatchlings may have been sub-normal in several respects; their small size, evident feebleness, the low numbers to hatch from each pod, and a high proportion of completely non-viable pods all pointed to this. These defects notwithstanding, dietary carotene had a profound effect on the growth and pigmentation of modified hoppers, and as it was of little evident consequence in the nymphal development of normal hatchlings, this clearly showed that modified eggs lacked or had unusually low amounts of carotene, and that the amounts present in normal eggs were generally sufficient to mask the expression of a dietary deficiency in subsequent nymphal development.

To some extent this information helps to reconcile the contradictory results obtained in previous work on the effect of carotene on the growth of *Schistocerca*. It will be shown later that the growth difference observed in modified *Locusta* is susceptible of various interpretations, but the fact that it occurred at all lends

substance to the hypothesis put forward in the introduction that, given ovarian carotene, its masking effect on subsequent dietary requirements (for carotene) might be more or less incomplete in those eggs having low concentrations, particularly if growth were generally inferior and development more protracted, as it was with the yeast-containing diets used in the early experiments. It is unfortunate that support for this hypothesis rests on information obtained mainly with *Locusta*, but, so far as they go, the results of one experiment with modified *Schistocerca* indicate a similar masking situation in both species.

The most striking effects of dietary carotene on modified *Locusta* hatchlings concerned coloration. Although rearing densities were such as to induce gregarious characteristics, these appeared only where carotene was present in the diet. Without it, the strong melanisation characteristic of gregarious hoppers failed to develop (modified hatchlings were all initially unmelanised), and in many cases a greenish-blue colour appeared in the integument, of a sort which might be expected of insectoverdin if deprived of its carotenoid component. So far as coloration was concerned, the effect of depriving *Locusta* of carotene was therefore to induce a tendency for *solitaria* facies to develop. The relative activities of modified hoppers were in accord with this interpretation, for those given carotene were markedly more active besides being strongly melanised. On the other hand, no morphological differences were noted in the hoppers, all appearing to be gregaroid (*i.e.*, with a flat pronotum), although adult morphometric data surprisingly showed, if anything, a general solitaroid tendency.

Modified *Locusta* hatchlings were able to complete nymphal growth without carotene, but mortality was greater and the adults obtained were smaller, in both sexes, than those allowed carotene. As differences in colour and activity were such as to indicate that deprivation of carotene tended to induce a solitaroid facies, it might have been the case that the difference in growth was a secondary reflection of this tendency. Solitary locusts are generally somewhat slower to develop than crowded, and it was observed that modified hatchlings without carotene on the whole took slightly longer to pass through each moulting period. The females of phase *solitaria* tend to be larger than those of phase *gregaria* at fledging, and the males, while differing less, differ in the reverse way (Gunn & Hunter-Jones, 1952). However, both the male and female adults obtained from modified hatchlings were smaller when deprived of carotene, and this suggests that a direct impairment of growth was involved irrespective of any possible secondary consequences of a general solitaroid tendency.

In the light of these results with modified *Locusta* hoppers, it is of interest to reconsider the effects on pigmentation which carotene has been observed to have in *Schistocerca*. Its most noteworthy effect concerns the vivid yellow body colour of gregarious fourth- and fifth-instar hoppers, for this is directly due to integumentary carotenoids (Goodwin, 1952) and cannot develop in the absence of dietary carotene (Dadd, 1957). However, in normal hoppers reared without carotene the body colour is not white, as it is in the early instars when so reared, but has areas of a faint yellow-grey, greenish-yellow or green colour. These are presumably caused by the mobilisation of the small reserves of carotene derived from the (normal) egg, and the general occurrence of a greenish component in these faint colours may be taken to indicate a tendency for small amounts of insectoverdin to be formed. *Schistocerca* hoppers showing these external pigmentation changes due to lack of dietary carotene usually had blue blood by the fifth instar. As the blue pigment has been shown to have similar electrophoretic properties to the blue component of the insectoverdin complex of green blood from grass-reared hoppers, it may be presumed that the prosthetic substance concerned is mesobiliverdin, the blue prochromogen of insectoverdin (Goodwin & Srisukh, 1951).

Dietary carotene further affected the coloration of normal *Schistocerca* hoppers

(and fledglings) indirectly, by a modifying action on the redox pigment insectorubin. In the absence of carotene the pink colour of the early instars and fledglings, due to free insectorubin in the hypodermis (Goodwin, 1952; Nickerson, 1956), failed to develop, although some bound insectorubin could be shown to be present by its appearance in the pink, free state on boiling (Dadd, 1960). Insectorubin is morphologically localised beneath the melanised areas of the cuticle in *Schistocerca* nymphs (Nickerson, 1956) and a metabolic relationship between the two pigments has been suggested (Goodwin, 1950). It was therefore of interest that in modified *Schistocerca* hoppers the absence of dietary carotene markedly impaired the production of cuticular melanin besides suppressing the pink colour of free insectorubin.

It may be argued that these consequences of carotene deficiency in normal *Schistocerca*, as in modified *Locusta*, afford some indication of a tendency in pigment metabolism towards the condition in the solitary phase, for in both cases melanisation tended to be impaired and mesobiliverdin was produced, and these are the characteristic features of *solitaria* pigment metabolism in both species (Goodwin & Srisukh, 1951). Green *Schistocerca* solitaries are further characterised by the almost complete absence of insectorubin (Goodwin, 1950), and it may be relevant in this connection that a lack of dietary carotene, while not suppressing the formation of insectorubin, always suppressed its appearance in the free state. All these nutritionally induced pseudo-solitaroid effects were more or less reversed by the inclusion of carotene in the diet. Even so, fully gregarious pigmentation was never achieved with either species under conditions of rearing density which should have ensured it (and did so when the food was grass). However, the failure of the fat-body ever to become yellow on synthetic diets indicated that the uptake of carotene from them was in some way impaired, and thus some degree of carotene deficiency was always present.

The implications of these carotene-dependent effects must now be considered in relation to the problem of the characterisation of phase in locusts, for while morphological criteria are of primary importance in this connection with adults, the phase status of nymphs is largely decided by visible pigmentation. The phase theory was developed to account for the fact that certain species of poly-morphic ACRIDIDAE exist in two extremely different but intergraded forms which can give rise to progeny of the opposite type, the mechanism responsible for this transformation being connected with the degree of crowding during development (Uvarov, 1921, 1923, 1928; Faure, 1932).

In its original form this theory has come under heavy criticism in recent years, exhaustively reviewed by Key (1950) and Kennedy (1956). Objections have been levelled at the early attempts to explain locust outbreaks too exclusively in terms of phase transformation, particularly in view of the inconsistencies which have emerged where different criteria have been used to define phase. While it is now recognised that various phase criteria are less interdependent than was at first thought, and require the cumulative conditioning of successive generations for their complete expression, it is generally accepted that degree of crowding is of crucial importance in their determination, although quite clearly genetic and environmental factors other than density must play some part in the result finally attained.

Here we are concerned primarily with those aspects of phase characterisation which involve coloration. The biochemistry of pigmentation in both *Schistocerca* and *Locusta* has been related to the external coloration of the phases (Goodwin, 1952). Briefly stated, the darker *gregaria* coloration which develops under crowded conditions of rearing is characterised by the production of integumentary melanin, while the uniform, generally paler and often green coloration of *solitaria* locusts reared in isolation is characterised by the absence of melanin and the production of a green pigment, insectoverdin.

Now while it is true that the extreme products of solitary and gregarious rearing are characterisable by qualitatively different pigments, this distinction is by no means absolute, and environmental conditions other than rearing density may effect great changes in the development of these same pigments. When the rearing temperature is very high, melanin is not formed in crowded hoppers (Goodwin, 1950; Husain & Ahmad, 1936), while a high concentration of CO₂ may be able to induce melanin formation in solitary *Schistocerca* (Husain & Mathur, 1936). Moreover, several authors have suspected that humidity, the quality of food and humoral malfunction may modify the type of coloration to be expected on grounds of density alone (Gunn & Hunter-Jones, 1952; Nickerson, 1956; Stower, 1959; Joly, P., 1951).

The particular interest of the pigmentation effects here shown to be related to the presence or absence of dietary carotene is that they simulated qualitatively the pigmentation characteristics of the *gregaria* and *solitaria* phases, respectively. In so doing they afford experimental evidence that nutritional factors can outweigh density-dependent effects in the determination of certain crucial phase criteria. This leads to the conclusion that, if phase is thought of as essentially those physical and behavioural manifestations characteristic of certain densities of crowding, the normally accepted phase criteria may be reversed. Alternatively, if phase is characterised primarily by particular physical and behavioural attributes (*i.e.*, colour and activity) rather than by the history of density during development, the implication is that characteristics of the *solitaria* phase may arise in gregarious conditions.

Neither of these conclusions can be accepted with satisfaction, more particularly as the highly artificial state of carotene depletion involved in the experiments on which they are based is unlikely to be met with outside the laboratory. There is no likelihood that the natural food of solitary locusts could ever lack carotene to the extent that was required to effect pigmentation experimentally; on the contrary, the *solitaria* facies has been observed to develop best when particularly succulent fresh food was provided (Faure, 1932; Gunn & Hunter-Jones, 1952; Okay, 1953; Nickerson, 1956), and such plant material is likely to contain high concentrations of carotene (Kohler, 1944). Moreover, the available data afford no indication of any difference in the over-all carotene content of solitary and gregarious locusts (Goodwin, 1949).

A more acceptable hypothesis of the nature of the action of carotene in these experiments may be sought in relation to the intermediary physiological events whereby in normal circumstances the degree of crowding of developing nymphs is enabled to regulate morphological characteristics and the production of particular pigments. This field of inquiry is, unfortunately, for the most part, a matter of speculation, but two aspects of it, concerning vision and humoral regulation, merit consideration, albeit speculative.

It has been suggested that vision may be an important factor in contributing to the mutual nervous stimulation which has been supposed to set in train the process of gregarisation when locust hoppers are crowded together (Chauvin, 1941; Kennedy, 1939, 1956; Key, 1950). If this is so, it might be expected that impairment of vision could be of significance as a solitarising agency. Now the photosensitive pigments of visual sensory cells are universally found to contain carotenoids as the active prosthetic group. In vertebrates, crustacea and molluscs vitamin A is involved, but, although a retinene-like pigment has recently been extracted from the eyes of house-flies (Bowness & Wolken, 1959), vitamin A itself has not hitherto been unequivocally detected in insects. In particular, it was found to be absent from *Schistocerca* (Fisher & Kon, 1959), and it has been suggested that astaxanthin might be the photoreceptor of locusts (Goodwin & Srisukh, 1949). However, as the presence of either vitamin A or astaxanthin would depend on the availability of carotene as a precursor, impairment of vision

might be anticipated in the absence of carotene, irrespective of the identity of the actual photosensitive pigment. The hypothesis of defective vision, entailing a tendency towards *solitaria* characteristics must, therefore, be borne in mind when considering the experimental results described in this paper.

The possibility that carotene may affect pigmentation by a mechanism related to humoral regulation is, perhaps, a more fruitful field of speculation, for hormones have been implicated in colour changes involving insectorubin and insectoverdin in several insects, among them locusts. Numerous studies (reviewed by Joly, P., 1945) show that in stick insects the changes involved are caused by the migration of pigments, analogous to the well-known phenomena in crustacea, but a humoral influence on pigment metabolism has also been demonstrated, and it is with this aspect of colour change that we are concerned.

Changes in pigment metabolism connected with the endocrine-regulated events of metamorphosis have been recorded in Lepidoptera. In certain species of *Papilio*, the colour of the pupa, green or brown, is determined in the larva shortly before pupation by environmental stimuli acting *via* a hormone from the prothoracic ganglion (Ohnishi, 1959). Green pupae lack melanin and possess a typical insectoverdin, whereas brown pupae contain astaxanthin and other oxidised carotenoids, have little mesobiliverdin, and are melanised. Ohnishi points out that these differences are somewhat similar to those which distinguish the phases of locusts, likewise determined by environmental stimuli acting *via* a humoral mechanism. A hormone-dependent colour change from green to red occurs in the larva of *Cerura vinula* (L.) shortly before pupation. The pigment concerned is an ommochrome (to which group of pigments the insectorubin of locusts belongs), and its production requires the presence of the prothoracic hormone. α -ecdysone (Bückmann, 1959). Premature reddening could be induced by decapitation in young larvae, and it is suggested that in the early instars the corpora allata (juvenile) hormone is of importance in maintaining the normal green coloration by affecting the competence of tissues to produce ommochrome under the influence of ecdysone.

In locusts, the corpora allata have been implicated in the endocrine system whereby the phase coloration of nymphs is determined, and in addition have been shown to affect the maturation colour of adults. The implantation of corpora allata from gregarious *Locusta* (adults or nymphs) into young *gregaria* nymphs caused a high proportion to develop green *solitaria* coloration (Joly, P., 1951; Joly, L., 1954), a change similar to that brought about in modified *Locusta* hatchlings by withholding carotene, if due allowance is made for the appearance of mesobiliverdin rather than insectoverdin in the latter case. A less marked tendency towards *solitaria* coloration followed implantation in *Schistocerca* (Joly, P., 1949, 1951); subsequent work confirmed this, and further showed that whether *gregaria* or *solitaria* were used to donate or receive implants, any changes in colour were always in the solitaroid direction (Nickerson, 1956). Cross injection of haemolymph was found to work in the opposite direction; that is, gregarious haemolymph changed *solitaria* hoppers (and implanted fragments of *solitaria* integument) in a gregaroid direction, but the reverse did not occur.

To account for these experimental results, Nickerson (1956) proposed a scheme involving the secretion of two hormones by the corpora allata, governing background and pattern coloration, respectively, and produced in variable ratio dependent on the density of rearing. Two hormones were held to be necessary if the autonomy of background and pattern pigmentation was to be maintained, but as, following the suggestions of Okay (1953) and Goodwin (1950), a metabolic relationship between the two systems was envisaged, it seems unlikely that autonomy could be complete. The postulate of a single hormone was rejected because it was held to offer no explanation of why corpora allata implants produced only changes in a *solitaria* direction, whereas injections of haemolymph produced

only changes in a *gregaria* direction. But this objection might be overcome by supposing that, were a single solitarising hormone concerned, its concentration in the blood must exceed a certain minimal level to be effective. For as all injection experiments would probably involve a reduction in the concentration of hormone compared to that in the untreated phase where its activity was apparent (*solitaria*), it can be envisaged that, irrespective of the phase of donors and recipients, the final concentration in the mixed blood resulting from such experiments could be so low as to be ineffective. The result would be, as Nickerson found, that recipients would never change in the solitaroid direction, but could (when solitaries) become more gregaroid.

An alternative hypothesis not requiring the attribution of additional hormones to the corpora allata may be constructed somewhat as follows. As mesobiliverdin and insectorubin both contain pyrrole structures, the suggestion has been made that they may be interrelated (Okay, 1953). If they are metabolically interdependent in the sense that one or the other tends to be synthesised (perhaps from a common precursor) with changes in the metabolic equilibria, and supposing that the gregarious phase is the more stable condition (Kennedy, 1956; Nickerson, 1956), it can be envisaged that excessive production of hormone from the corpora allata might, by sufficiently shifting the metabolic equilibrium, lead to the preferential production of mesobiliverdin at the expense of insectorubin, thus inducing a tendency for solitaroid coloration to develop. If melanin production is linked to the presence of insectorubin, this tendency would at the same time restrict melanisation. In this connection the dependence of melanisation on a suitable redox environment for the action of tyrosinase (Mason, 1955; Buck, 1953) and the possibility that the redox pigment insectorubin may be involved in oxidative reactions (Bellamy, 1958) may be relevant. As these same changes in pigmentation follow deprivation of carotene, it is reasonable to suppose that a shift in the metabolic equilibrium similar to that brought about by heightened corpora allata secretion then occurs. In so far as carotene affects several pigments to which it bears no obvious chemical relationship, an indirect function of the sort here suggested would seem to be a necessary feature of any hypothesis of its action.

Although the foregoing interpretation involves consideration of only one hormone, this is not to say that others may not be implicated. The more stable gregarious condition referred to must itself be dependent on the appropriate activity of a balanced endocrine complex; for such is now held to regulate the over-all metabolism of all insects. If we refrain from postulating hormones additional to those commonly held to occur in most insects, the scheme here outlined amounts to the proposition that *solitaria* coloration in the nymphs of locusts is caused by excessive production of juvenile hormone. It is of considerable interest in this connection that Kennedy (1956) and Carlisle & Ellis (1959) have adduced a variety of grounds for supposing that phase *solitaria* is a more 'juvenile' form than phase *gregaria*.

Brief reference must be made to an effect on adult pigmentation that the corpora allata have been shown to have in *Schistocerca*. In this species gregarious males, when mature, become bright yellow, due to the appearance of β -carotene in the cuticle (Goodwin, 1952). When allatectomised, this change, together with certain other features of the maturation process, does not occur (Loher, 1958, 1959). In so far as yellowing in the adult male is a feature of the gregarious phase only, the corpora allata hormone might in this case be held to act in an opposite phase direction to that found in nymphs. However, in the context of adult insects, the function of this endocrine organ is primarily concerned with sexual maturation (Bodenstein, 1953), and this particular colour phenomenon is clearly associated with the events of maturation. It is, perhaps, not surprising to find then that in the adult context where the juvenile hormone is generally no

longer concerned in the maintenance of juvenile features, that it should influence coloration in a gregaroid rather than a solitaroid manner.

It was mentioned in the introduction that all attempts to encourage maturation and oviposition in *Schistocerca* reared on synthetic diets had been unsuccessful. Males failed to become yellow, copulation rarely occurred and the few females which deposited eggs dropped them around the cages instead of forming pods in the tubes of sand provided for this purpose. On a few occasions when adults of *Locusta* were kept they copulated readily shortly after fledging, but again male yellowing failed to occur and the occasional oviposition was haphazard. The failure of males to become yellow is readily understood in terms of poor uptake of carotene from synthetic diets, and it is a matter for surmise whether the over-all failure in reproduction under these circumstances might be indicative of a more fundamental requirement for carotene in the processes of reproduction. To pursue this point out of the realm of speculation it will first be necessary to discover why carotene is so poorly utilised from synthetic diets.

Summary.

A method of obtaining locust eggs which could be expected to be deficient in carotene is described. It involved rearing a parental generation on an artificial diet which, because of certain pigmentation abnormalities it induced, was probably itself deficient in carotene.

Using crowded hatchlings of *Locusta migratoria* (L.) from eggs thus modified, it was shown that they were sensitive to dietary carotene, whereas hatchlings from normal eggs were indifferent. The absence of carotene was marked by inferior growth, lessened activity and most notably by an extremely different coloration in both hoppers and adults. Without carotene, melanisation was absent or greatly reduced and in many cases the integument developed a greenish-blue colour. With carotene, heavy melanisation occurred in the hoppers, and the colour of the adults was characteristically gregarious.

It was concluded that in normal eggs the amount of carotene present is usually sufficient to mask the expression of a dietary deficiency during nymphal growth, but that with normal eggs having low amounts of carotene, a dietary deficiency might become apparent as it did with modified eggs.

It is suggested that the blue colour of modified *Locusta* hoppers reared without carotene is due to mesobiliverdin, a prochromogen of insectoverdin, the green pigment of the *solitaria* phase of locusts. Insectoverdin itself could not be formed because it contains carotenoids, and these were absent from the diet. The effect of extreme deprivation of carotene is therefore to induce solitaroid tendencies, notably in regard to colour (suppression of melanin and production of mesobiliverdin) but also in regard to activity.

Crowded hoppers of *Schistocerca gregaria* (Forsk.) reared on synthetic diets lacking carotene usually had turquoise-blue blood by the fifth instar. It was shown, by the electrophoresis of yellow, green and blue bloods in parallel, that the blue chromoprotein of blue blood is the same as the blue chromoprotein of green blood. Its prosthetic pigment must therefore be mesobiliverdin.

The abnormalities of coloration which arise in crowded *Locusta* and *Schistocerca* when deprived of carotene are normal in the solitary phase. Moreover, they resemble those abnormalities of coloration consequent upon the implantation of additional corpora allata into gregarious hoppers. The implications of this are discussed in relation to the validity of phase criteria, and an attempt is made to relate these findings to hypotheses on the humoral regulation of phase.

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SOME EXPERIMENTS TO DETERMINE THE METHODS USED IN HOST-FINDING BY THE TSETSE FLY, *GLOSSINA MEDICORUM* AUSTEN.*

By R. F. CHAPMAN
Birkbeck College, London.

9.2.

A number of field experiments have been carried out on the host-finding of tsetse flies of the groups of species of *Glossina* related to *G. morsitans* Westw. and *G. palpalis* (R.-D.) (Buxton, 1955) but there is no published information on any member of the group of *G. fusca* (Wlk.). *Glossina medicorum* Aust., a member of this group, is relatively common in parts of Ghana near to Accra (Chapman, 1960) so that it was possible to carry out a series of laboratory and field experiments on the methods employed by this species in discovering its host. The experiments were concerned only with the perception of a host from a distance, not with the reactions of the fly when close to the host, and so they relate primarily to the visual and olfactory senses.

Methods.

In most of the laboratory experiments the fly was placed in a 1-litre conical or flat bottomed flask which, in turn, was placed inside an observation box. This consisted of an octagonal box of hardboard 100 cm. across and 60 cm. high with the whole of the inside painted white and illuminated by a lamp in the roof. One wall of the box was hinged as a door and this and the opposite wall contained a small slit at eye level through which observations could be made. By this means it was possible to watch the fly without disturbing it. The box was mounted on a table which had a hole one foot square cut out of the centre with another small table 11 in. square fitting into it. There was a small gap between the tables so that the fly, which was placed on the small inner table, was not disturbed by vibration of the outer table and, as the floor of the room was of concrete, the inner table was virtually free from vibration.

The flies were kept in an air-conditioned room at 26°C., but before experiments they were given some time to become acclimatised to the temperature of the room in which the experiments were to be carried out. This temperature was not controlled, but the bulk of the experiments were carried out within the range 27–32°C. The range of temperature for each individual experiment is quoted separately. Light intensity measured directly upwards at the floor of the observation box was 20 ft.-candles, and relative humidity in the air-conditioned and the experimental rooms varied from 60 to almost 100 per cent.

The flies were tested singly so that there was no question of one active fly stimulating others to move. This is important since it is most unlikely that any mutual stimulation occurs in the field. In the laboratory experiments the activity of each fly was recorded manually on a kymograph and the results are expressed in terms of the average time spent moving in each half-minute of the experiment. Equal numbers of males and females in similar stages of hunger were used in all the experiments but, except in the case of those recently fed, there were no very marked or consistent differences in the results so that all the stages of hunger of both sexes are considered together in the results. There was

* This work was carried out at the Biological Research Institute, University College of Ghana.

some tendency for males to be more active than females but the difference was not great. Each fly was tested only once on each day except when it was fed; on these days tests were carried out before and after feeding. The flies were fed on guineapigs every three or four days and attempts to feed them more frequently were not generally successful. Guineapig blood was found to be satisfactory for keeping the flies alive but, although some lived for more than three months, very little breeding occurred on this diet.

One factor emerging most clearly from the results was the great variability in the behaviour of the flies. Because of this, comparison of the activity of flies in different experiments is not valid and only differences within a single experiment can be considered as real.

Vision.

It was found in the field that *G. medicorum* was not attracted to traps or to stationary objects but it came in large numbers to a moving Land-Rover (Chapman, 1960). The aim of the following experiments was to determine whether or not this might be related to vision rather than smell, since tsetse are known to respond to the smell of vehicle exhausts (Napier Bax, 1937). It was necessary first to establish that they did respond to a moving object without the interference of smell and then to determine if their reactions were definitely directed towards the object rather than being mere random movements.

Laboratory experiments with a moving object.

The responses of *G. medicorum* to a moving object were first tested on a small scale in the laboratory. For this work a miniature electric locomotive, running on a circle of rails within the observation box, was fitted with a mat black screen

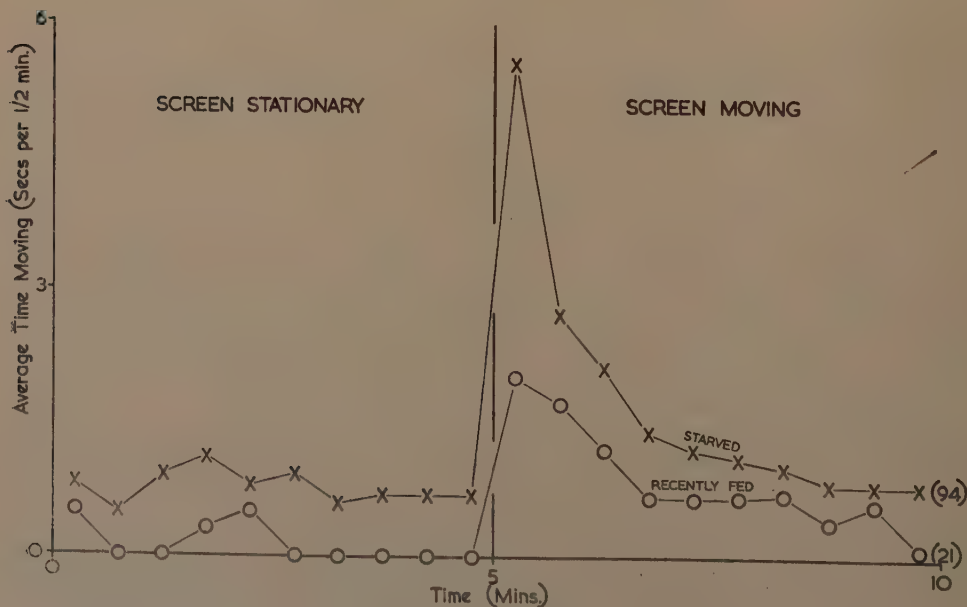


Fig. 1.—Response of *G. medicorum* in the laboratory to a moving screen. In this and subsequent figures the vertical lines indicate the points at which the screen started or stopped and figures in parenthesis to the right of each curve show the number of experiments carried out in each case.

(30 × 15 cm.) so that only the screen was visible to the fly in the centre of the box. The screen was about 30 cm. away from the fly and it was possible to control its speed and direction of movement from outside the observation box. The fly was placed in a stoppered flask on the centre table and it is believed that it was effectively free from the influence of outside smells or vibration.

In the first series of experiments, carried out at temperatures between 29 and 32°C., the screen was kept stationary for the first five minutes and was moving continuously round the track for the second five minutes. There was a very marked increase in the activity of the flies when the train started to move (fig. 1), but activity fell off rapidly and after four minutes of movement had reached the low level observed before movement started. Hungry flies were slightly more active than recently fed ones but the speed of the train, whose angular velocity varied from 30 to 50°/sec., was not important (Table I).

TABLE I.

Response of flies to a screen moving at different speeds.

Angular velocity (°/sec.) approx.	Number flies tested	Average time active (secs./min.)									
		Minutes before movement					Minutes after start of movement				
		5	4	3	2	1	1	2	3	4	5
30	65	0.6	1.4	1.2	1.2	0.6	6.5	5.3	3.9	1.7	2.8
40	30	0.9	0.2	0.1	0.2	1.1	8.6	7.7	4.9	3.8	2.8
50	94	1.3	2.0	1.7	1.3	1.4	8.2	3.5	2.3	1.8	1.6

A comparable series of experiments was carried out in which the black screen was hidden from the fly by a cylinder of white card just inside the track. This was to confirm that there was no response to the vibration of the moving screen, and fig. 2 shows that this was the case; activity was no greater in the second half

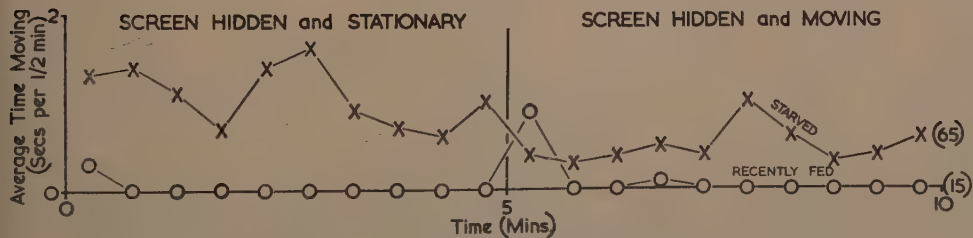


Fig. 2.—Laboratory experiments to confirm that the flies do not respond to the vibration of a hidden but moving screen.

of the experiment with the screen moving than it had been in the first half with the screen stationary.

A response to the moving screen was also obtained when the timing of the experiment was altered so that the screen was stationary for one minute, moving

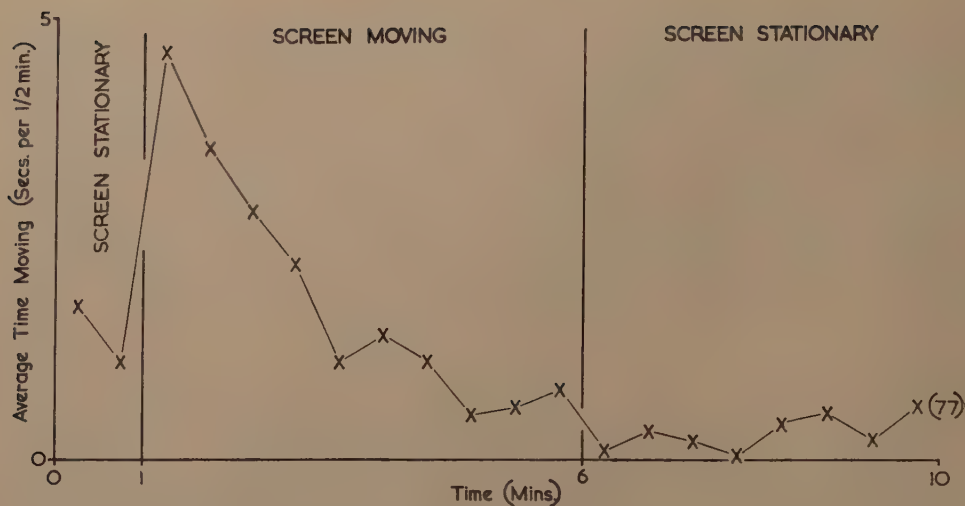


Fig. 3.—Response of flies in the laboratory to a screen stationary for one minute, moving for five minutes and then stationary for four minutes.

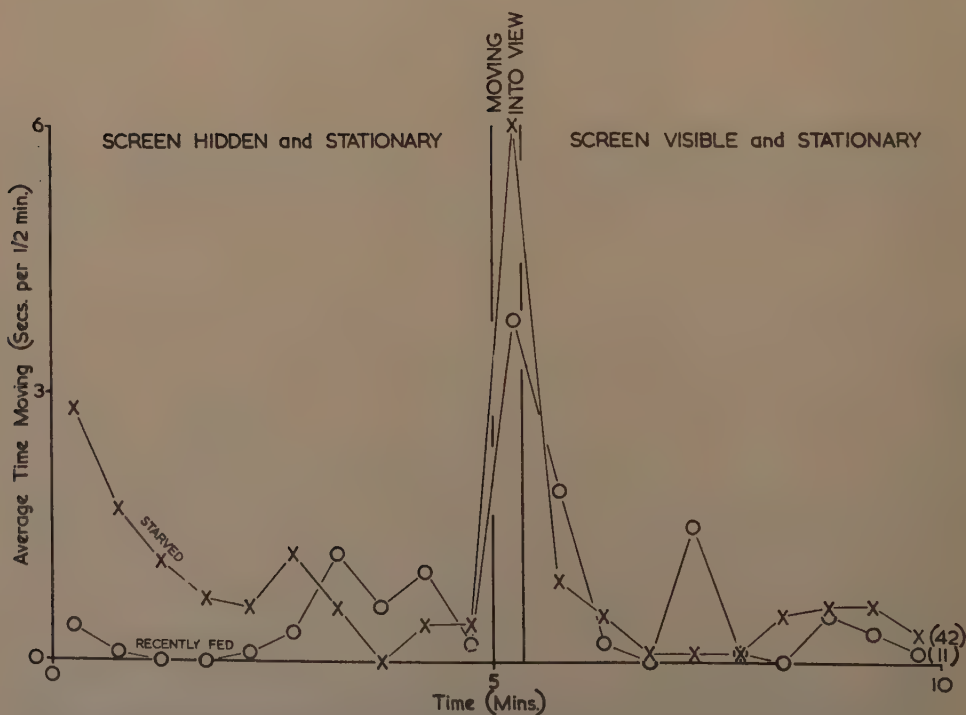


Fig. 4.—Response of flies in the laboratory to a screen at first stationary and hidden, which is then moved into view and left stationary.

for five minutes and then stationary for a further four minutes (fig. 3). A peak of activity at the beginning of the movement indicates that the timing of the experiment was not important (*cf.* fig. 1) and also that the response was clearly not in any way related to the length of time spent by the fly in the flask.

In another series of experiments the screen was hidden stationary within a tunnel of white card for five minutes. It was then moved slowly into the view of the fly and left stationary for a further five minutes. There was a sharp peak in the activity of the flies when the screen first appeared but this was followed by an immediate reduction to the original level (fig. 4). Only in the first half-minute after the appearance of the screen was activity greater in the presence of the screen than in its absence and the peak of activity is attributable to this sudden appearance. This suggests that the mere presence of the screen did not affect activity and that activity was induced only by a moving object and not by a stationary one.

Finally, experiments were carried out in which the screen was kept stationary for the first five minutes and then alternately moved and kept stationary for successive 30-second periods over the second five minutes. The activity of the flies increased each time the train moved and decreased each time it stopped (fig. 5) and, although the increase in activity became less each time, the fall off was less rapid than in the earlier experiments with continuous movement.

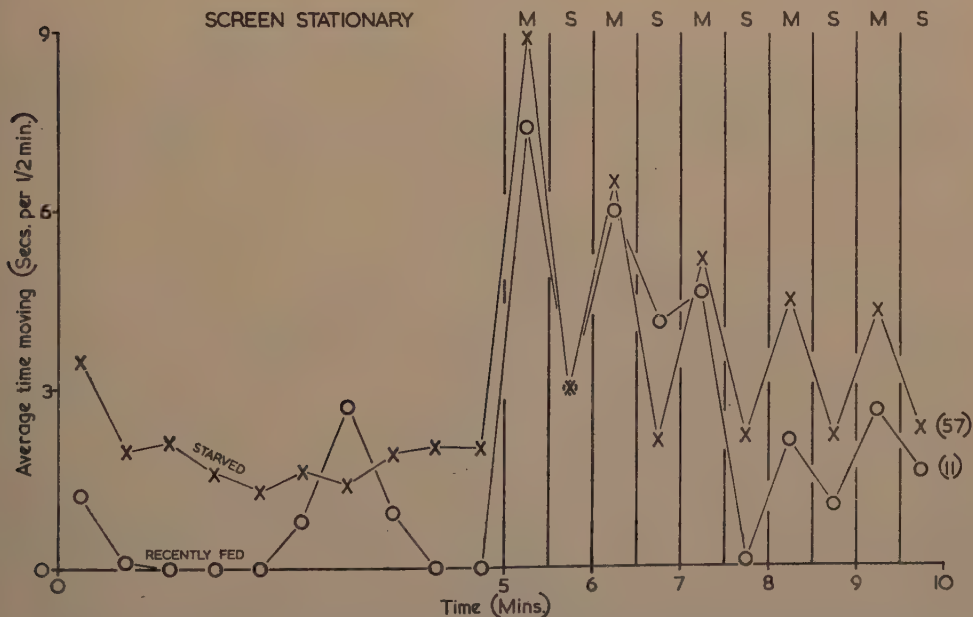


Fig. 5.—Response of flies in the laboratory to a screen that was moving (M) and stationary (S) for alternating 30-second periods for five minutes following a stationary period of five minutes.

Field experiments with a moving object.

Having established in the laboratory that *G. medicorum* did respond visually to a moving object, comparable experiments were carried out on a larger scale in the field. As before, the fly was placed in a 1-litre conical flask inside the observation box but in this case one of the side panels of the box was removed so that part of an open grass field was visible to the fly. The field was free from

bushes and sloped gently upwards from the box to a fairly flat skyline. The grass was very short so that the field of vision presented to the fly within the box was relatively uniform.

The moving object was a screen of black corrugated iron approximately four feet long and three feet high mounted about six inches above the ground on small wheels. By means of a rope attached to one end the screen could be

TABLE II.

Response of *G. medicorum* to a moving screen 25 ft. away in sunny and cloudy conditions.

Light	Number flies tested	Percentage of flies moving										
		During time intervals before screen moving					While screen moving and in view	During time intervals after screen moving				
		5	4	3	2	1		1	2	3	4	5
Clear sun ..	49	28	33	28	33	37	53	33	35	37	39	39
Cloudy ..	41	27	24	29	34	27	51	27	27	27	27	15

The lengths of the time intervals, numbered 1-5, are related to the time for which the screen was in view.

drawn across the field of vision of the fly at any distance required. No account was taken of wind speed or direction since the fly was enclosed in a stoppered flask and was, therefore, not influenced by air movements or smells. Activity was recorded on a kymograph for five minutes before the screen was drawn across the field of vision, for the time during which the screen was in view and for a

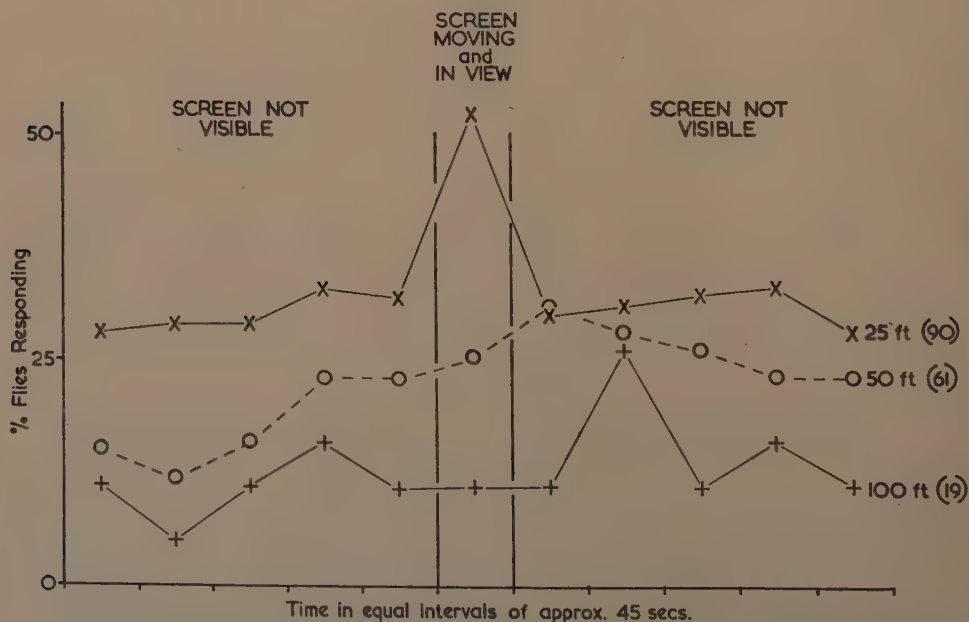


Fig. 6.—Response of *G. medicorum* in the field to a moving screen at different distances from it.

further five minutes after the screen had disappeared. The results are calculated in terms of time intervals equal to the time for which the screen was in view. This varied from 30 seconds to one minute according to the distance of the screen from the observation box and the activity of the assistant pulling it. In each time interval a fly is regarded only as being active or inactive so that in figs. 6 and 7 the results are expressed as percentages of flies moving in each time interval.

With the screen at a distance of 25 ft. from the fly there was no substantial difference between the results (Table II) obtained under a clear sky and those obtained in cloudy weather (light intensity from the direction of the screen varied from 20 to 75 ft.-candles). Accordingly, in fig. 6 the results from all weather conditions are pooled. The flies did show a response to the screen at 25 ft., although only 52 per cent. became active. At greater distances there was no suggestion of a response (fig. 6).

The response of *G. medicorum* was compared with that of *G. morsitans* which was collected in northern Ghana. Under similar conditions of light intensity (20–60 ft.-candles) and temperature (27–34°C. for both species) *G. morsitans* showed a very clear response to the moving screen from a distance of 150 ft. (fig. 7). From fig. 7 it appears that there was no response at 200 ft. but it was

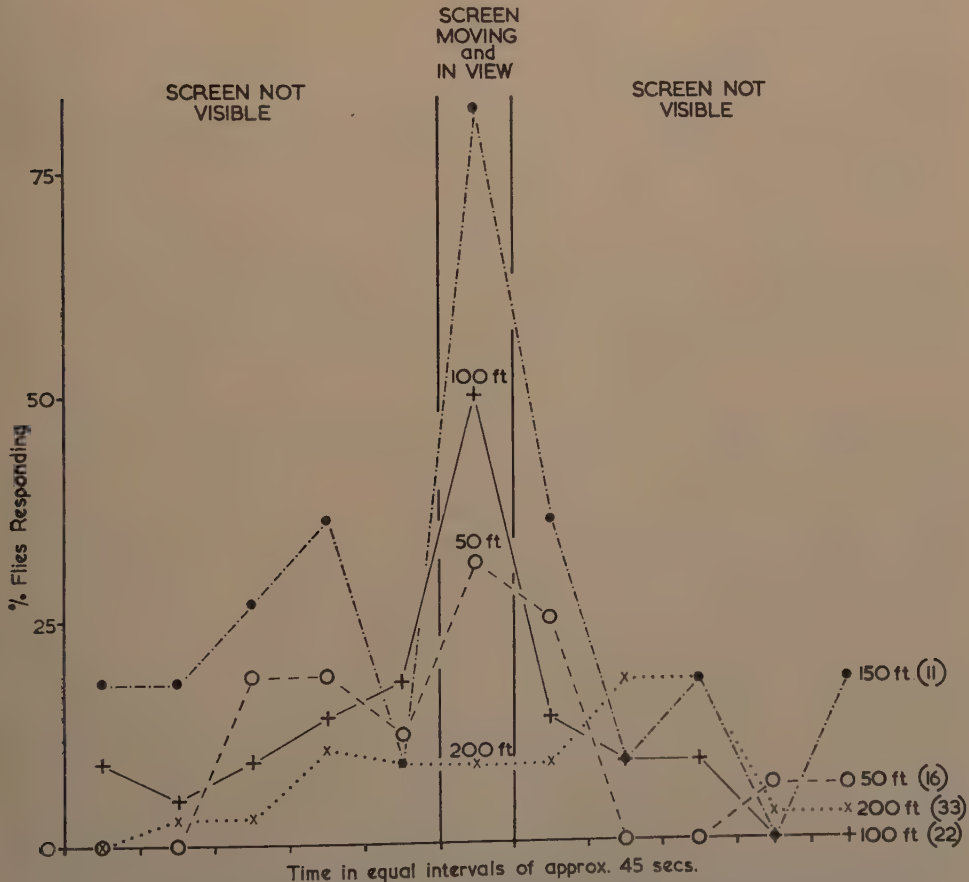


Fig. 7.—Response of *G. morsitans* in the field to a moving screen at distances up to 200 ft.

almost certain from the behaviour of some individual flies that they had seen the screen. For instance, a fly which was cleaning might suddenly stop and turn to face the screen in a manner which strongly suggested that it had seen the movement.

Of the 49 experiments on *G. morsitans* with the screen at a distance of 150 ft. away or less, 29 were carried out in sunlight and 20 in cloudy conditions. Sixteen of the flies responded to the screen in sunlight and eight when it was cloudy and, since this difference is not significant ($\chi^2 = 0.8$), it is concluded that the vision of *G. morsitans* was not materially affected by the presence or absence of sunlight up to a distance of 150 ft.

Movement towards an object.

From the previous experiments it seemed that, at least at short distances, *G. medicorum* was stimulated to activity by a moving object. A further series of laboratory experiments was then carried out to determine whether or not the flies were attracted to the object or merely flew randomly.

A small loop of thread was attached with rubber solution to the mesonotum of flies to be tested. From this loop a longer thread could be passed to a hook in the roof of the observation box, the thread being long enough to reach from the centre of the roof diagonally to the bottom edge of the walls. A fly attached in this way was gently placed on the centre of the floor and, while in many cases it took off immediately, over a period it was possible to make a number of observations on the responses of the fly to a miniature screen (30×15 cm.). The screen was kept stationary for five minutes and then, if the fly had not already moved, was set in motion for a further five minutes or until such time as the fly took off. If at the end of ten minutes the fly had not moved, the experiment was abandoned. The temperature in the box during these experiments varied from 24 to 29°C.

The fly took off during the first five minutes with the screen stationary in 115 experiments and in 67 of these it landed on the screen, while in the other 48 it landed on the walls of the observation box. Since the area of the walls, not counting the floor and roof, was some 32 times the area of the screen this result clearly represents a highly significant response to the screen ($\chi^2 > 1,000$, $0.001 > P$).

○	1	3	2	○	○
○	2	○	2	2	1
7	2	9	7	3	3
1	3	4	4	○	3
3	3	16	7	5	4

Fig. 8.—The numbers of tethered flies which settled on different parts of a small screen in the laboratory.

After five minutes the screen was set in motion and this resulted in a movement by the fly in 114 cases. The fly landed on the walls of the box on 64 occasions and on the screen on 50, sometimes pursuing it as it moved round the track. The movement towards the screen was again highly significant ($\chi^2 > 500$, $0.001 > P$) so that these results suggested a movement towards it irrespective of whether or not it was moving. The smell of the screen could not be excluded in these experiments but there seems to be no reason why its smell should have been more attractive to the fly than that of the observation box itself and it is considered that the response was essentially a visual one.

When the orientation of the fly was noted after it had landed on the screen it was found to be facing upwards on 17 occasions, downwards on 6 and sideways on 4. In addition the position of each fly on the screen was noted in most cases and there appeared to be a distinct preference for landing on the lower half of the screen and also in the centre rather than at either end (fig. 8).

Smell.

It seemed fairly certain that vision could play some part in host-finding by *G. medicorum*; other experiments were carried out to see if smell might also be important.

Laboratory experiments.

In the initial experiments on smell, tsetse flies were tested singly in a Thorpe olfactometer with 'clean' air, drawn from outside a window and over banks of activated charcoal to remove other smells, passing down one arm and air drawn through a chamber containing a guineapig down the other. Each test lasted five minutes and, although in most cases the fly did not move at all, 38 movements into the arms of the olfactometer were recorded. Only 17 of these were towards the source of the smell, the other 21 being towards the clean air. The difference was not significant ($\chi^2 = 0.4$, $P > 0.1$) and clearly did not suggest any response to the smell. The temperature range in the experiments was 26–33°C.

A second series of experiments was carried out with the fly inside the observation box and in a flask through which air could be drawn. The air supply either came from outside the window as clean air or through a tube which was strapped with its opening along the side of a goat in an enclosed room. This second air supply was assumed to be carrying the smell of the goat. The activity of each fly was recorded on a kymograph and each was subjected to five minutes of clean air followed by five minutes of air carrying the smell of the goat.

In 22 experiments it was found that there was less activity with the smell than without it (an average of 27 seconds active in five minutes compared with 37 seconds in the clean air). This suggested that the smell might be reducing activity but when the experiment was run using clean air continuously for ten minutes there was again slightly more activity in the first five minutes than in the second (28 secs. and 22 secs. in 14 experiments). There was no significant difference between the two sets of experiments ($\chi^2 < 1$, $P > 0.1$) and the difference in the two parts is attributed to the long time taken by the flies to settle down in a stream of moving air. The temperature range was 27–32°C.

This experiment was repeated but with the smell produced by bubbling air through very dilute acetic acid. Five minutes of clean air was followed by five minutes of smell and then a further five minutes of clean air. The smell took about 5 seconds to reach the flask when the supply was turned on but rather longer to clear when replaced by clean air again. In this case there was a very marked initial response to the acid followed by a falling off in the response (fig. 9) although this was less rapid than in the visual experiments, but it is not clear if this really was a response to smell or if it was initiated by the common chemical sense (Roeder, 1953, p. 545) since acetic acid is certainly an irritant to humans.

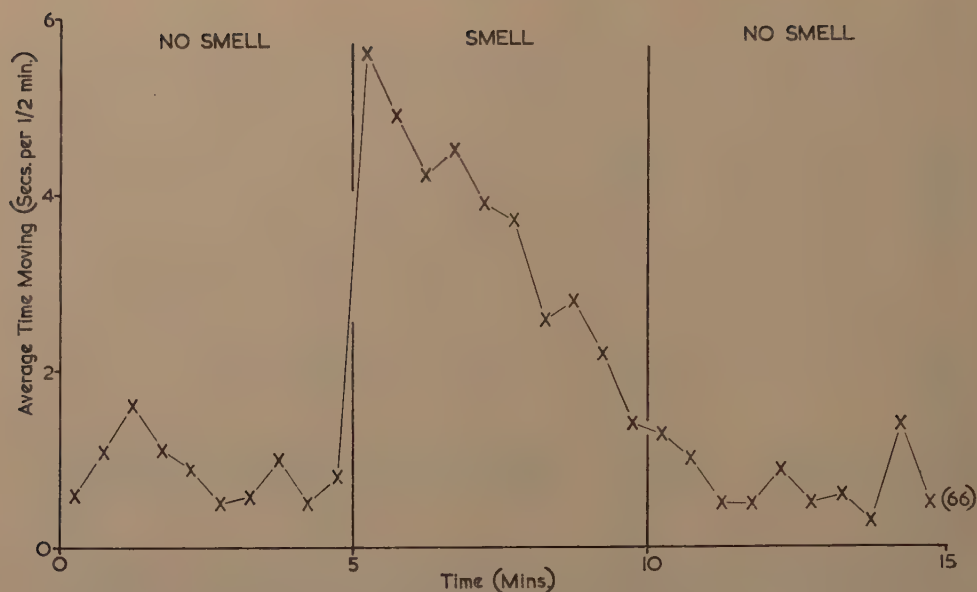


Fig. 9.—Response of flies in the laboratory to stimulation by vapour of acetic acid.

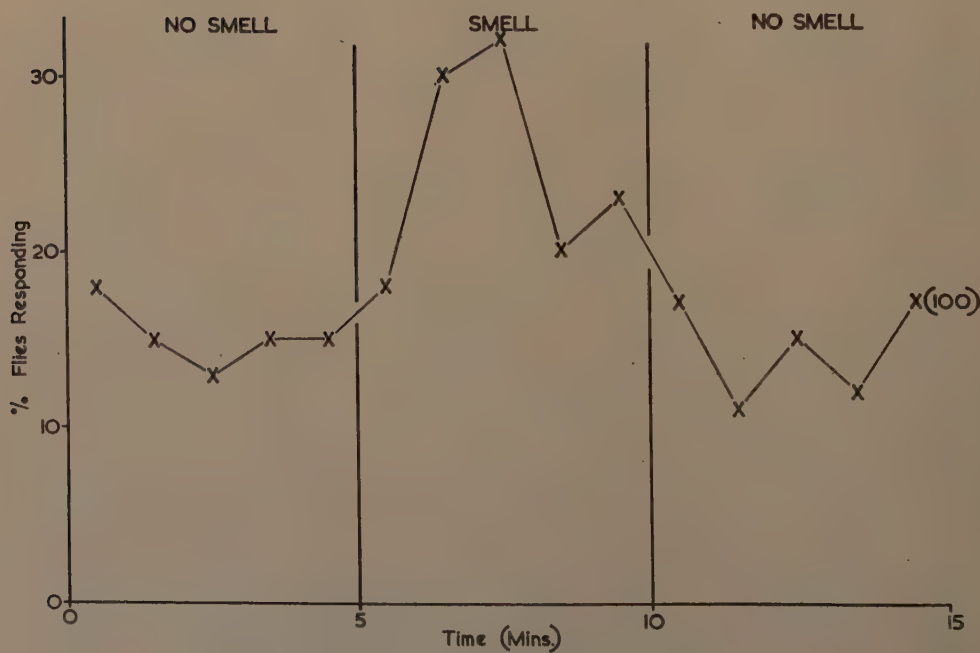


Fig. 10.—Response of flies in the field to the smell of cows.

Field experiments.

The laboratory experiments showed conclusively that *G. medicorum* responded to acetic acid but this could not be regarded as significant in the finding of a host in the field, particularly since no response was obtained using guineapigs and goats. The failure of these experiments prompted a more natural experiment in the field, using cows.

The fly was put in a cage of mosquito gauze on a wire frame 15cm. \times 10cm. \times 5cm. and the cage was placed in the field on a table screened from the sun and from the observer who sat close behind making observations through a peep-hole. A screen of black canvas six feet high, arranged as if round three sides of a rectangle with a long side of 20 ft. and two ends of 10 ft., was fixed to convenient trees, and the table, with the fly on it, was placed in the centre of the open rectangle. Thus the visual field of the fly consisted of the black canvas screen on three sides and a white card screen on the fourth side and forming a roof above it. On the other side of the canvas screen were four cows under the control of an assistant and completely invisible to the fly. A wind vane was just visible to the observer behind the screen so that the assistant could be directed to move the cows to a position directly upwind of the fly, in order that their smell reached it, or to keep them well away from due upwind, so that their smell did not reach the fly.

The screen was set up on the boundary of a field which was empty except for the cows and the assistant. The main smells reaching the fly were therefore likely to be that of the cows *plus* the assistant and that of the observer sitting close up behind a screen of card. The observer was in fact downwind of the fly but so close up that his smell must have been apparent. Nevertheless a clear-cut response to the cows was obtained in the experiment and this tends to suggest that *G. medicorum* does not respond to the smell of man. This is supported by the failure of *G. medicorum* to attack a man on foot in habitats where it is known to be common (Chapman, 1960).

In each experiment each fly was recorded as active or inactive in each minute for five minutes in the absence of any smell, five minutes with the cows due upwind and another five minutes without any smell (taking that of the observer for granted). During most experiments the wind speed was less than 5 m.p.h., and the results of the few carried out at higher wind speeds did not vary from the rest so that all the results are considered together. Temperature varied from 25 to 30°C.

There was a very marked response by the flies to the smell of the cows (fig. 10), 30 per cent. of the flies being active compared with only about 15 per cent. in the absence of the cows.

Laboratory experiments on orientation to wind.

G. medicorum is stimulated to become active by the smell of a possible host but if it is also to determine the direction of the host by smell it must either be able to follow a gradient of smell or it must be able to orientate to the wind carrying the smell. Under field conditions of air turbulence it is very unlikely that stable gradients of smell can exist so that host-finding by following a gradient is most improbable. The following experiments were carried out to determine whether or not *G. medicorum* could orientate to air movements.

The experiments were carried out in a wind tunnel mounted in the observation box. The working section of the tunnel was a perspex cylinder 33 cm. long and 7.5 cm. in diameter. Air passed into and out from this by symmetrical conical ducts at either end, where honeycomb grids were fitted to help smooth the air flow. The source of air movement was a fan which could be mounted at either end of the tunnel so that the air flow could be reversed.

A piece of 2-amp. fuse wire was fixed with rubber solution to the mesonotum of the fly being used and the free end was passed through a fine glass tube and slightly bent over at the top to prevent it slipping out of the tube. The latter passed through a cork which fitted into a hole in the centre of the wind tunnel so that the bottom of the cork was flush with the inside of the tunnel. Thus the fly was suspended in the centre of the wind tunnel on the end of a piece of fuse wire which was free to rotate in the glass tube and which could also ride up to a considerable extent under the lifting power of the fly in flight. In this way the fly was able to orientate to the air movements although its position in the tunnel was fixed.

No anemometer was available to measure the wind speeds but these were standardised by the use of a swinging plate which could be suspended in the tunnel. The theoretical wind speeds, assuming no friction at the hinge, were calculated from the deflection of the plate and, while they are certainly lower than the true values, the values obtained are of the correct order of magnitude.

A card with black and white stripes transverse to the length of the wind tunnel was placed beneath the working section so as to present a definite visual pattern to the flies. During the experiments the orientation of each fly was recorded at 5-second intervals and the experiment was continued until the fly stopped flying or was seen to be merely hanging on the wire flapping its wings and not supporting its own weight. At first each fly was subjected to alternate one-minute periods of still and moving air but in later experiments air movement was continuous.

A strong upwind orientation by the flies was apparent at all wind speeds (Table III), although orientation at higher wind speeds was slightly better than

TABLE III.

Orientation of flies at different wind speeds.

Wind speed (m.p.h.)	Number flies tested	Total time flying (min.)	% of time flying		
			into wind	with wind	across wind
<5	58	159.75	90	3	7
>5	20	198.75	99	0	1

at lower ones. A similar result was also obtained using flies blinded by painting their eyes with black cellulose paint. In this case 11 blinded flies flew for a total of 54 minutes and for 94 per cent. of this time they were headed directly upwind.

In the absence of a wind, flight was reduced and less steady, but in 64 minutes and 50 seconds of flying in still air by normal flies only 11.2 per cent. of the time was spent in spinning, with the flight completely lacking orientation. With blinded flies in still air, 15 flies flew for 17 minutes, 45.6 per cent. of which time was spent spinning. This suggests that vision did play some part in orientation even though blinded flies could orientate accurately to an air current.

Discussion.

The experiments on vision indicate that in the laboratory and at short distances in the field *G. medicorum* was induced to become active by dark moving

objects. Jack (1939) also found this in experiments with *G. morsitans* using a cylinder rotating outside the cage but unfortunately he does not give full details of the work. In field experiments, Napier Bax (1937) showed that *G. swynnertoni* Aust. could see oxen moving at a distance of 450 ft. Thus this species and *G. morsitans* are able to see moving objects at some distance whereas the experiments indicated that *G. medicorum* did not respond to objects more than 25 ft. away. This difference may be related to the difference in habitat of the species concerned. *G. morsitans* and *G. swynnertoni* are savannah species living in habitats where the trees are generally well separated and the visibility is good. *G. medicorum*, on the other hand, inhabits high forest or thicket where visibility is generally very restricted and good distance vision would be of very little significance.

The failure to find any effect of sunlight on the response to the screen by both species is surprising since Napier Bax (1937) recorded better distance vision in sunlight and Barrass (1960) has shown that *G. morsitans* has a tendency to land on the sunlit side of a screen rather than on the shaded side.

It is noteworthy that the presence of a stationary screen did not promote activity and this may be related to the fact that *G. medicorum* is not attracted to normal stationary tsetse traps but may be taken in large numbers in a moving vehicle (Chapman, 1960). The movement of tethered flies in the laboratory towards the stationary screen is thought to have resulted from their excited state following handling and is, therefore, not strictly comparable with conditions in the field or with the other experiments where time was allowed for the flies to settle down.

The response to the screen when it was alternately stopped and started for short periods may also be of significance when the normal habitat of this species is borne in mind. In the field it is quite likely that a possible host would move sporadically and might also repeatedly disappear behind bushes to reappear a little later. Continued flight when the host had disappeared could easily lead to the fly losing contact with it so that any tendency to stop when the host disappeared and wait until it reappeared or started moving again before resuming flight is clearly advantageous.

Sufficient flies were not available to carry out field experiments on the movement towards conspicuous objects but the laboratory experiments gave a clear positive result. Movement towards dark objects must be common in other species, since otherwise tsetse traps could not be effective, and the failure of *G. medicorum* to come to such traps is probably related to its general low level of activity. Nash & Davey (1950) have commented on the difficulty of catching this species because of its sluggishness, and it appears that it becomes active only when strongly stimulated, as by a moving object.

Buxton (1955) pointed out that experiments on smell were complicated by the difficulty of contaminating the apparatus with human smell. This may have accounted for the failure of the laboratory experiments in which a guinea pig or a goat was used as the source of smell but it seems more likely that their unsuitability as hosts was the real reason. The experiments using acetic acid, comparable with the work of Hughes (1957) on *G. palpalis*, may be taken to indicate only that *G. medicorum* will react to the vapours of some chemicals, but the field experiments indicate that smell might be important in host finding. Napier Bax (1937) obtained a similar response to the smell of oxen with *G. swynnertoni*.

Various field experiments have been carried out in which tsetse flies were attracted to animals hidden behind screens but these cannot be regarded as indicating the importance of smell since the flies may be attracted primarily to the screen and only secondarily, if at all, by the smell of the animal. This is commonly overlooked and even Buxton (1955) says "... it is easy to exclude

sight by using screens." The fallacy of this was shown during the field experiments on smell when numbers of TABANIDAE, *Atylotus* sp., *Tabanus tacniola* P. de B. and *Ancala fasciata* (F.), were collected from the large black canvas screen behind which the observer was sitting. At other times these flies were commonly collected biting the cattle but in this instance they apparently preferred the large canvas screen to the four cows which were standing nearby in full view.

The experiments of Vanderplank (1944), in which greater numbers of flies were attracted to scented than to unscented screens, show that movement may be directed towards the source of smell, and Jack (1939) released marked individuals of *G. morsitans* from a box and obtained a 50 per cent. recovery upwind but no recoveries downwind. In this instance it is impossible to decide whether the smell of the observer carried on the wind, the wind itself, or the two factors combined was responsible for the movement and the orientation. It is possible that in this case and in *G. medicorum* the actual smell might be the activating influence while the resulting flight is orientated upwind.

No attempt was made to evaluate the relative importance of vision and smell in host-finding by *G. medicorum*. It may well be that in the natural habitat of this species smell is the initial activating stimulus. Orientation into the wind would then bring the fly to the vicinity of the host with the final approach depending on the fly seeing the host and moving directly towards it.

Summary.

Field and laboratory experiments were carried out in Ghana on the tsetse fly, *Glossina medicorum* Aust., to discover the senses used by the fly in finding its host. In laboratory experiments on vision a model locomotive with a black screen attached to it was used as a 'host'. The flies were stimulated to move by the movement of the screen. When the screen was stopped and started for short periods the increase in activity became less each time the screen was put in motion but the fall off was less rapid than in experiments with continuous movement. The possible significance of this is discussed in the light of the normal habitat of the species in the field.

In the field, *G. medicorum* did not respond to a moving object more than 25 ft. away but *G. morsitans* Westw. showed a good response at distances up to 150 ft.

Tethered flies were attracted to the model screen and showed a preference for landing on the lower edge near the centre.

Laboratory experiments using a guineapig and a goat as sources of smell produced no response from the flies but a strong reaction was obtained with the vapour of dilute acetic acid. In field experiments, using cows which were not visible to the fly, a very marked response to the smell of the cows was obtained. Orientation to a source of smell probably involves orientation to air movement and *G. medicorum* showed a strong upwind orientation in a wind tunnel even when blinded.

It is concluded that *G. medicorum*, which lives in thicket or forest where visibility is poor, probably responds initially to the smell of a potential host. An upwind orientation would then bring it to the general vicinity of the animal and the final approach might be visual.

Acknowledgements.

I am indebted to Mr. J. E. Biles for assistance in the construction of the wind tunnel and to Mr. J. W. Charter for the calculation of wind speeds. Mr. Gabriel Dza was responsible for maintaining the flies in the laboratory and also assisted in the field experiments.

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STUDIES ON THE DISPERSION AND SURVIVAL OF *ANOPHELES GAMBIAE* GILES IN EAST AFRICA, BY MEANS OF MARKING AND RELEASE EXPERIMENTS.

By M. T. GILLIES

*East African Institute of Malaria and Vector-borne Diseases,
Amani, Tanga, Tanganyika.*

(PLATES I & II.)

Despite their importance as vectors of disease, we know very little at present about the movements or flight range of most tropical mosquitos. In Africa, small-scale observations were made by De Meillon (1937) and Adams (1940) on *Anopheles gambiae* Giles in Northern Rhodesia, who found maximal flights of one to four miles. Apart from this work, our knowledge is mainly limited to the observations of those concerned with control of breeding sites in small towns, whose general impressions may be summed up by the view that, provided the surrounding areas are not totally unpopulated, a controlled zone of one to one and a half miles renders a township relatively malaria-free. More precise information is essential therefore to understanding not only particular problems of malaria control but also wider aspects of the biology of this important species.

The study of the expectation of life of mosquitos has received much stimulus from recent advances in age-grouping techniques, as developed by Soviet workers. However, the technical difficulties have not yet been solved for most of the vectors of malaria in the tropics, and complementary information on their survival in nature obtained by other methods cannot fail to be of value. An attempt was accordingly made to study the movements and survival under natural conditions of *A. gambiae* in the humid coastal belt of Tanganyika. The method employed was the release and recapture of marked, insectary-reared mosquitos. The first aim was to determine flight range, with reference to general behaviour rather than to occasional flights of exceptional length made by individual insects. Secondly, we set out to study dispersion in relation to age, and ultimately, if it was found that there was little or no progressive loss of mosquitos by emigration as the population aged, to obtain some picture of longevity and mortality. These objectives were partially fulfilled, and this paper is an account of the two different marking methods used, of the flight range of recaptured mosquitos, the effect of prevailing winds, the relationship of age to dispersion, and the pattern of survival of marked mosquitos.

Methods and material.

Labelling technique.

By painting.—As already reported, Gillies (1958a), the topical application of paint was used as a marking method for the study of the duration of the first gonotrophic cycle in *A. gambiae*.

A field insectary was established in the centre of the experimental area. The rearing technique used was that devised by Shute (1956). The eggs used were derived from locally caught females, with occasional supplements of eggs from the colony at Amani. On emergence, the adults were isolated and offered sugar solution. Releases were made three times a week, the mosquitos at the time of

marking being 16–40 hours old, except for those marked on Monday mornings, which included some that had emerged on the Friday evening.

Artists' poster paint, mixed with a little water to the right consistency, was applied with a micro-loop made from gauge .0024 plated copper wire. These lengths of wire were fixed to the ends of matchsticks with candle wax, each forming a delicate instrument with which it was impossible to exert more than the lightest pressure. The mosquitos were caught five at a time in a sucking tube, into which just sufficient chloroform vapour was sucked or poured to knock them down. The moment they were anaesthetised they were tipped out on to a strip of cork, gently flipped over with a dissecting needle so as to lie dorsal side up, and the cork was placed under a wide-field, low-power magnifier for painting. By gently touching each mosquito in the centre of the mesonotum with the tip of the loop, it was possible to lift them up just off the cork. A slight tap of the hand holding the loop would cause the insect to fall back again, leaving a discrete spot of paint at the site of contact. The cork was transferred to the release cage until the mosquitos recovered, which took between 5 and 30 minutes. As the paint dried in about a minute, it was essential to make certain that the mosquitos did not roll over and become stuck to the cork. A useful modification to the technique was the use of a dilute solution of Teepol. If the loop was dipped into this and then into the paint, a smaller spot remained on the insect than if the paint alone was used.

Fifteen different colours or blends were used which, at the rate of three releases a week, allowed for an interval of five weeks before the same colour recurred. Only females were marked. An output of 1,000 mosquitos a week was aimed at, which worked out in practice at between 200 and 400 a morning. Under field conditions, two assistants could handle about 150 mosquitos in an hour, although in the main laboratory considerably faster rates of painting could be achieved. One mosquito was heavily painted, pinned, and kept as an example of the exact colour used on that day. All recaptured mosquitos were examined individually with a hand lens, and any marked specimens compared with the reference series. Assistants did the preliminary sorting, but with the exception of one small series, the actual matching of the colours was always checked by myself. It was found that certain colours in the cream or buff range had to be avoided, since they could be confused with the spots of excreta occasionally ejected by mosquitos after knockdown and accidentally transferred to the thorax from one specimen to another when lying in the storage tins before sorting.

With radioisotopes.—Radioactive tracers have been widely used for marking mosquitos, and in the experiments described here no departures from established practice were introduced, apart from the serial use of two different isotopes. The eggs were derived more or less equally from wild-caught females, from a recently established colony of the local strain of *A. gambiae*, and from a long-established strain originally obtained from Kenya. The isotopes used were ^{32}P , supplied as a solution of orthophosphate, and ^{35}S , supplied as sulphate, both obtained from the Radiochemical Centre, Amersham. The solution containing isotope was introduced into the breeding pans two to three days before pupation, in the third or early fourth instar. A dose of 5 microcuries of ^{32}P , or of 15 microcuries of ^{35}S per litre in the breeding pans gave rise to high enough levels of radioactivity in the emerging adults to be easily detected. No attempt was made to do any quantitative estimations of radioactivity, and the only counter in use was a simple monitoring instrument for checking contamination after handling active solutions.

Labelled mosquitos were detected by means of autoradiography. The insects to be tested were attached to cellulose tape, stretched over a numbered grid (see Pl. I), details of the catch being attached to the end of the tape. These were applied to strips of X-ray film (Ilford, Industrial G, 29 × 280 mm.) in a dark-room, and normally exposed over two nights, that is for about 40 hours. To distinguish

insects labelled with the two different isotopes, use was made of the technique of energy discrimination, as described by Gillies (1958b). In most instances there was no possibility of confusing the diffuse fogging, produced by mosquitos labelled with ^{32}P , with the sharp images of those labelled with ^{35}S . These differences are illustrated in Plate II. But in every instance in which there was any doubt, the mosquitos were re-exposed on double layers of film when, in contrast to the effect of ^{32}P , those with ^{35}S only left an image on the strip of film in immediate contact with them. Use was also made of the observation by Duncombe (1959) that, when using X-ray film which is normally coated with emulsion on both sides, objects labelled with ^{35}S leave an image on *one side* only of the film.

Evaluation of labelling technique.

In addition to simplicity and cheapness, a satisfactory marking method must be recognisable throughout the life of the insect, and be without obvious harmful effects. Both these criteria were studied, with the following results.

Persistence of labels.—It was not easy to check the permanence of the mark in mosquitos that had been painted, except in caged specimens. Once dried, poster paints show no tendency to crack or chip, and it seems unlikely that in nature the mesonotum of a mosquito would be subjected to either abrasion or wetting. It should be pointed out that the mesonotal scales, themselves extremely delicate objects, are seldom rubbed or missing in wild-caught mosquitos at the time of capture. In fact, the greatest risk to any ornamentation on a mosquito, whether natural or applied, lies in its treatment after capture, and steps to minimise this risk were incorporated in our recapture routine.

With radioactive tags, there are two main sources of loss of activity that could lead to difficulty or doubt in recognising long-lived specimens. These are decay, and elimination of labelled material during formation and deposition of eggs.

It might be thought that decay would be an important factor when using ^{32}P , since, with a half-life of 14.3 days, three-quarters of the radioactivity will have been lost by the end of a month. However, ^{32}P is taken up so readily by developing mosquitos, and its effect on photographic plates so intense, that radiation is easily detected in marked specimens up to 7–8 weeks after emergence. ^{35}S has a half-life of over 12 weeks, and there is, correspondingly, relatively little loss through decay.

Loss of radioactivity through the eggs is less easy to assess. There was substantial loss after the first few egg-batches, particularly in the case of radiol sulphur. The rate of loss is likely to fall off in older, less fertile females, but it was not possible to follow this in the laboratory. In females recaptured in the third or fourth week after release, doubtfully positive specimens were not encountered. However, the possibility of missing feebly radioactive females remained and, to minimise this risk, the exposure time of all catches was extended to 3–4 days during periods when old markings might have appeared in the catches.

A further practical point should be mentioned. With ^{35}S , internal absorption of low-energy beta-particles can be an important source of loss of detectable radiation, particularly in females distended with blood. When spreading the mosquitos out on the cellulose tape for testing, it was necessary to squash each specimen and also to apply very firm pressure to the film holders to make certain that the insects were in close contact with the surface of the film.

Perhaps the best illustration of the over-all effect of these sources of loss is provided by the autoradiographs shown in Plate II, in which actual recaptures of old marked mosquitos are compared with those of freshly emerged specimens. This shows very clearly both the reduction in radioactivity of the older mosquitos and its recognisable persistence at an advanced age.

One needs also to be reassured that no radioactivity is passed on through the eggs to act as an unintended label in the next generation. In laboratory tests

it was found that batches of eggs from females labelled with ^{32}P showed up faintly on X-ray film when first laid; but in the fourth-stage larvae, and adults reared from them, no radioactivity could be detected. With ^{35}S not even the eggs affected the film.

Biological side-effects.—Preliminary tests on the effects of labelling on the survival of mosquitos were carried out in the laboratory. Since the application of paint involves some handling of the mosquitos, as well as the administration of anaesthetic, it was to be expected that this might affect their longevity. The pigment used in any of the paints might also have been specifically toxic to mosquitos. Survival tests using insects marked with paint alone, or treated with Teepol with or without paint, or subjected to anaesthetic without painting, all resulted in a slightly higher mortality during the first 24 hours after marking; but, beyond that age, no difference in survival rates could be detected between any of these categories of mosquitos and those used as controls.

When using radioisotopes, there was virtually no handling of the mosquitos and, apart from unknown defects in rearing conditions, the only factor that could have any adverse effect was the presence of radioactivity. Survival tests in the laboratory on over 1,100 mosquitos gave the following results: mean age of controls, males 10.3 days, females 10.0 days; ^{32}P at 5 microcuries per litre, males 9.9 days, females 10.5 days; ^{35}S at 15 microcuries per litre, males 10.3 days, females 10.9 days. Thus it is clear that rearing in these solutions, at the dosage used, had no adverse effect on the subsequent survival of the adults. The labelled mosquitos were also fully fertile, and normal development of their progeny was observed.

Validity of experimental method.—Since it was planned to study the dispersion of mosquitos at all ages, it was essential to employ a marking method that would persist throughout the life of the insect. Some of the techniques that have been used in the past, such as dusting, or spraying with dyes, have been primarily intended for short-term experiments, and were not thought suitable for the present work. For this reason the topical application of paint was chosen, despite the extra handling involved. The method is particularly useful for small-scale releases where insectary facilities or opportunities for collecting larvae in nature are limited. Moreover, with careful blending and matching of colours a wide variety of different marks can be employed, such as are required when daily variations in behaviour are being studied.

The use of radioisotopes has the obvious advantage that large numbers can be released without handling. Its main limitations are those of expense and the difficulty of distinguishing different batches of marked insects. This is particularly serious where serial releases are planned and the exact age of the recaptures is required to be known. Both disadvantages were largely overcome by the autoradiographic technique used in these experiments.

A further word should be said about the two uncertainties mentioned above; namely, the possible harmful effects of painting, and the risk that the older isotope-labelled females were being missed. The latter possibility was very much in the front of our minds, and any strips of mosquitos that produced doubtful shadows on the film—and they were quite common—were re-exposed for longer periods. In no case did any turn out to be due to radioactivity. Any remaining doubts about the validity of the techniques used are further dispelled when the results of the two methods are compared. As is recorded below, the average ages at recapture in the parallel series of releases of painted and isotope-labelled mosquitos were identical. Thus, one must postulate either that the respective failings of the two methods matched each other exactly, or that they were of negligible importance. The latter seems by far the most likely explanation.

There remains one outstanding limitation in the experimental method adopted: the use of artificially-reared insects. This difficulty is almost insuperable with a

mosquito like *A. gambiae*, whose small, scattered, temporary breeding sites make it virtually impossible to collect adequate numbers of wild larvae. Consequently, as regards flight range, we cannot be quite certain that a natural population would have behaved in the same way, although the differences may not be great. But, when it comes to longevity, there is fairly good evidence from the incidence of malaria infection among the recaptured females that their span of life was unnaturally short. And as described below, estimation of mortality rates must be confined to the pattern of survival after release, that is to changes in mortality with age.

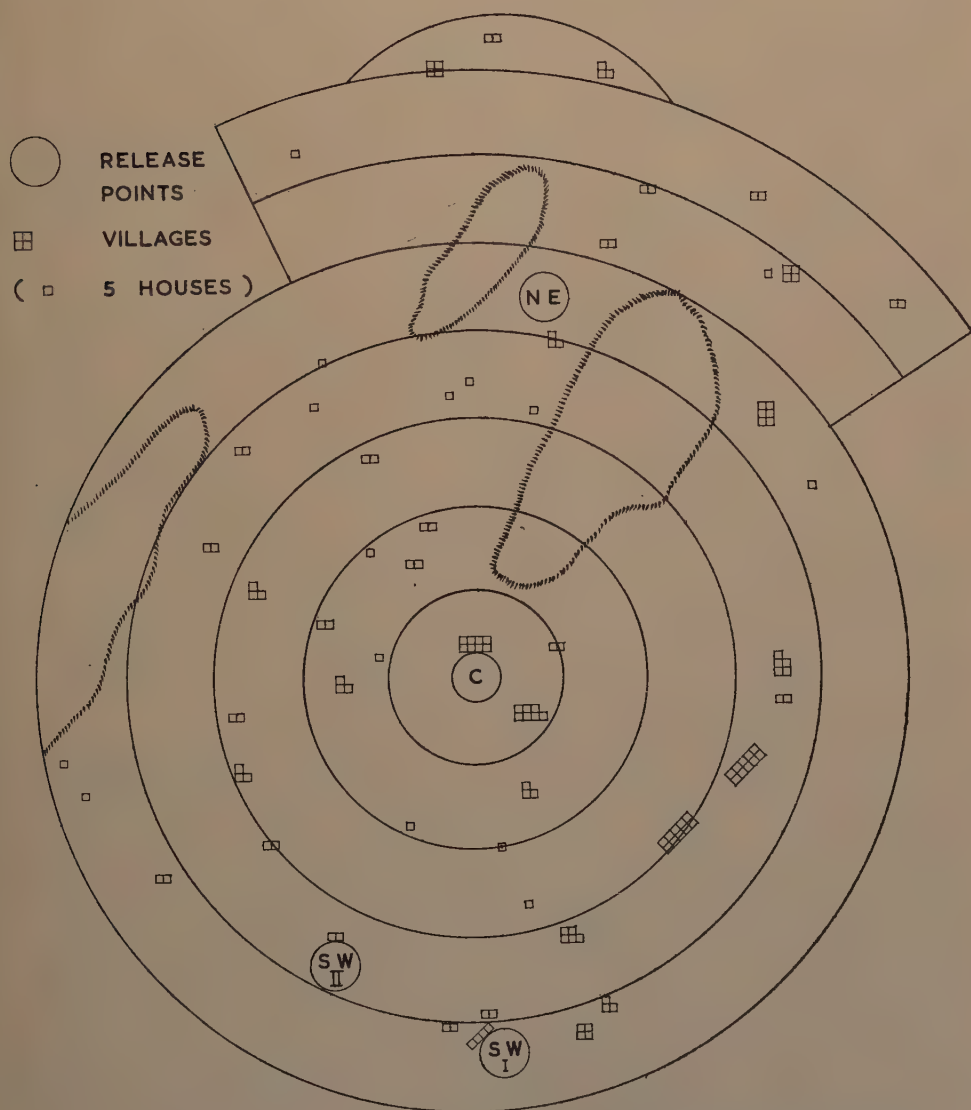


Fig. 1.—Sketch map of experimental area to show distribution and density of houses. The concentric circles are at quarter-mile intervals.

Release and recapture procedure.

Experimental area.—The area chosen for the experiments lay in the well-watered foothills of the Eastern Usambara Mountains. Accounts of the region have already been published by Gillies (1954, 1955). The area is moderately densely populated, and the people live in small villages or scattered family groups of houses. The distribution and density of houses in the experimental area, which consisted of a circle of radius one and a quarter miles, with an extension in one sector up to one and three quarter miles from the centre, is shown in fig. 1.

Releasing procedure.—The main bulk of the larvae pupated over a period of three days. In the experiments with radioisotopes, pupae were collected from the rearing pans and transferred to distilled water in waxed paper cups, at a density of 100 per cup. Ten to fifteen cups were put into each release cage, which was fitted with a sliding lid, and sugar-water was provided for the adults when they emerged. A count was made afterwards of dead pupae, trapped adults and dead adults remaining in the cages, from which the total number released was calculated. The sex ratio was found to approximate to 1:1, males tending to be more numerous on the first day of emergence. Adults emerged 24 hours after pupation, and were released as soon as practicable. About one-third were released within 18 hours of emergence, one-half during the second 24 hours, and a small number during the third day of adult life.

Four different points within the experimental area were used for the releases. The central release point was an experimental hut with wide, open eaves. The peripheral releases were made either from unoccupied and partly broken-down huts, or from box shelters of the type normally used for the capture of outdoor-resting mosquitos. The cages were put in position on ant-proof trays at around midday, and the lids carefully slid off. The few mosquitos that were disturbed by this action would fly out and settle on the adjacent walls. But most stayed inside the cages until the last hour of daylight when, after a period of mounting activity and of brief preparatory flights towards the failing light, continuing up till 10 to 20 minutes after sunset, a mass exodus through the eaves took place. A certain amount of predation occurred at this time. On one occasion geckos were seen patrolling the eaves and picking off the mosquitos that rested there before their final departure. On another, a Salticid spider, caught in the vicinity of the release cages, was found to be strongly radioactive when tested. On several evenings, mosquitos were seen streaming out from the release points and forming typical swarms in adjacent open spaces. Of 300 males netted and tested from one such swarm, 267 were found to be labelled.

Recapture routine.—Catching stations were maintained in one house out of five once a week within the one-and-a-quarter-mile radius of the experimental area. In addition, catches were extended out to a mile and three quarters in one sector, referred to from now on as the 'extension area', at a frequency of one house in five once a fortnight. Mosquitos were collected by spray-catching, that is to say, cotton sheets were spread over all horizontal surfaces and the room then sprayed with 0.1 per cent. pyrethrins in kerosene. Only bedrooms were used for the catches, and since in some houses there were several rooms, only one of which was sprayed, the fraction of the total house population collected from each catching station varied quite widely. Some elasticity in the choice of catching stations was allowed so that the actual house chosen in each group of five was not necessarily the same every time.

There were four spray teams for the main area, each consisting of two or three men, and one for the extension area, operating on a strict routine five days a week. Each had its own sector, and the villages visited on any particular day were as far as possible spread over the different parts of the sector. The total number of occupied houses within the one-and-a-quarter-mile radius fluctuated round 600, 120 of them being used at catching stations in each week. In the extension area

there were 12 catching stations in operation during the latter half of the experiments. The catches were maintained continuously throughout each series and for one month after the last release regardless of the place of release. Thus, apart from other factors, the chance of recapture at any particular point remained constant throughout the experiments.

Distribution of release points in relation to recapture area.—Releases were initially all made from the centre of the area. But after the first two series of experiments it became apparent that catches would have to be extended beyond the mile-and-a-quarter radius originally planned. The resources available did not permit an extension of the area up to two miles without a drastic reduction of the proportion of houses used as catching stations, and the recapture rate. So, to avoid this difficulty, it was decided to leave the recapture area as it stood, and to shift the release point to the perimeter instead. In this way, while those mosquitos that flew in one general direction would leave the area almost at once, the others would be available for recapture at any distance up to two and a quarter miles from the point of release. Two peripheral release points were accordingly set up near the north-eastern and south-western perimeter, and releases made alternately from the different points. After a short period it became necessary to move the south-western point to another village about half a mile away. Hence, four different release points were used, as shown in fig. 1. Releases of painted mosquitos were alternated more or less regularly from one point to another. The isotope-labelled insects were released in nearly equal numbers from the centre and from the periphery.

The peripheral releases raise some problems of interpretation when the density of recaptures in relation to distance flown is assessed. The difficulties are most readily to be comprehended by reference to fig. 2, which shows, in a diagrammatic

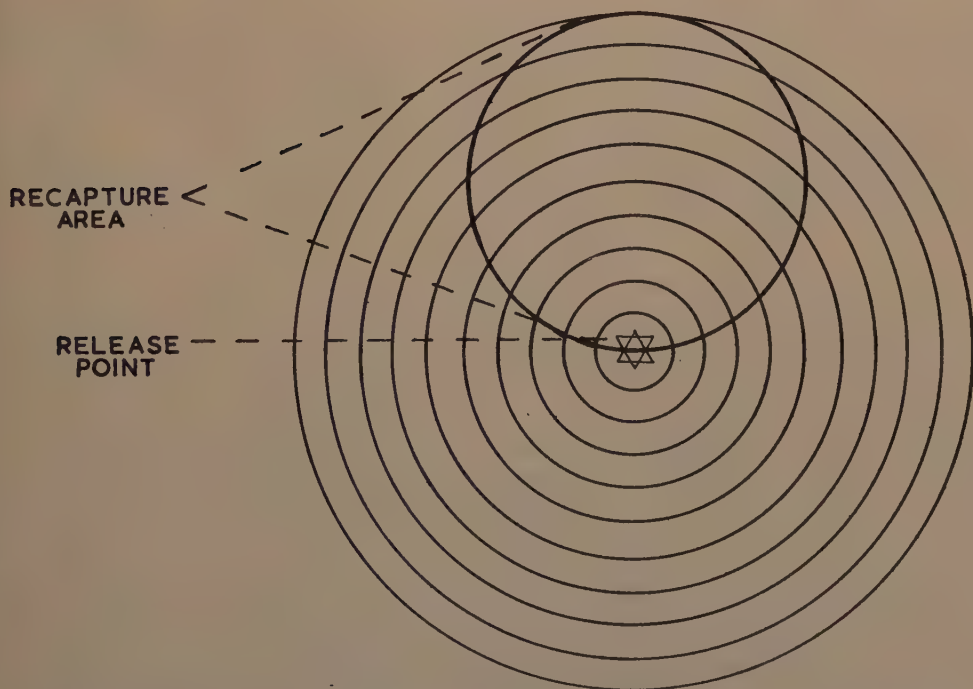


Fig. 2.—Diagram to show geometric relationship of recapture area to peripheral releases.

TABLE I.

Summary of results of all releases and recaptures.

Period	Method of labelling	Numbers released	Sex of releases	Trapping range	Proportion of houses used as catching stations (%)	Numbers recaptured		Per cent. recaptured		Total number trapped	
						Males	Females	Males	Females	Males	Females
March-June, 1956	Paint	3500	Females	600 yd.	27	—	66	—	1.9	—	14750
Jan.-Feb., 1957	Paint	3200	Females	$\frac{1}{2}$ mile	67	—	121	—	3.8	—	6100
March-July, 1957	Paint	13800	Females	$1\frac{1}{4}$ miles	20	—	55	—	0.4	—	32300
Oct., 57-Oct., 58	Paint	49250	Females	$1\frac{1}{4}$ – $2\frac{1}{4}$ miles	20	—	324	—	0.7	—	95500
Total	Paint	69750	Females			—	566	—	0.81	—	
July-Oct., 1958	Isotopes	8550	Males, females	$1\frac{1}{4}$ – $1\frac{1}{2}$ miles	20	36	64	0.8	1.5	1230	—
Jan.-June, 1959	Isotopes	54150	Males, females	$1\frac{1}{4}$ – $2\frac{1}{4}$ miles	20	120	233	0.4	0.9	14810	65350
Total	Isotopes	62700	Males, females			156	297	0.5	0.95	—	—
Total ..	All releases	132450				156	863			16040	214000

way, the geometry of the problem. From this diagram it is clear that, with each increase in range from the release point, the recapture area covers a smaller proportion of the potential area of dispersion at that range. For example, the whole of the $\frac{1}{4}$ -mile zone round the north-eastern release point was covered by routine catches; whereas only about one-seventh of the $2\frac{1}{4}$ -mile zone was included in the recapture area. It follows that this factor must be taken into consideration in estimating the effect of distance on density. The appropriate correction factors have been calculated geometrically, and are set out in the second column of Table VII.

Results.

Summary of releases and recaptures.

The results of all the releases are summarised in Table I. Of the successive series, the first was of a preliminary nature to test the painting technique and to find out what sort of recapture rate would be obtained. The second was designed to study the duration of the first gonotrophic cycle, and catches were accordingly concentrated in the vicinity of the release point. This resulted in a relatively high recapture rate, made up for the most part of young mosquitos (see Table III in Gillies, 1958a). In all the later series the recapture routine remained constant and the numbers of recoveries largely depended on the siting of the release points, whether in the centre or the periphery of the experimental area. In the case of the isotope-labelled releases, the recapture rate of the males was approximately half that of the females, a difference that presumably reflected the less domestic resting habits of male mosquitos.

Dispersion.

For the analysis of dispersion, only those series of releases in which the recapture area extended up to $1\frac{1}{4}$ miles and over are considered. The recoveries of marked mosquitos from the central releases, in which, as already explained, catches were made in all sectors up to a range of $1\frac{1}{4}$ miles and, for part of the period, up to $1\frac{3}{4}$ miles in the sector that included the extension area, are shown in Table II. The table gives the total numbers recaptured and the percentage of the total at each range, so that we are considering here the mean dispersion up to a distance of $1\frac{1}{4}$ miles, regardless of age. Within these limits it will be seen that recaptures of both sexes fell off steadily with increasing range, although there

TABLE II.

Recaptures of marked mosquitos from central release point.

Range (miles)	Numbers recaptured				Per cent. recaptured			
	Painted females	Isotope females	Isotope males	All females	Painted females	Isotope females	Isotope males	All females
0- $\frac{1}{4}$	64	65	59	129	36	38	55	37
$\frac{1}{4}$ - $\frac{1}{2}$	39	45	19	84	22	26	18	24
$\frac{1}{2}$ - $\frac{3}{4}$	30	31	12	61	17	18	11	18
$\frac{3}{4}$ -1	32	14	10	46	18	8	9	13
1- $1\frac{1}{4}$	12	17	7	29	7	10	7	8
$1\frac{1}{4}$ - $1\frac{1}{2}$ *	—	1	—	1	*Extension area: incomplete series, catches made on one quarter of perimeter only.			
$1\frac{1}{2}$ - $1\frac{3}{4}$ *	1	1	—	2				
$1\frac{3}{4}$ -2*	—	—	1	—				
Total	178	174	108	352				

is a marked concentration of males round the release point. The regression of density on distance for the recaptures of both sexes plotted on a semi-logarithmic scale is shown in fig. 3. Regression coefficients for males and females in the isotope releases are -0.21 and -0.17 , respectively. The same coefficient for all recaptured females is -0.16 . If one assumes that dispersion beyond the limits of the experimental area continued at the same rate, it is found (by extrapolation from the regression line corresponding to -0.16) that 78 per cent. of the mosquitos flew less than one mile, 18 per cent. from one to two miles, and only 4 per cent.

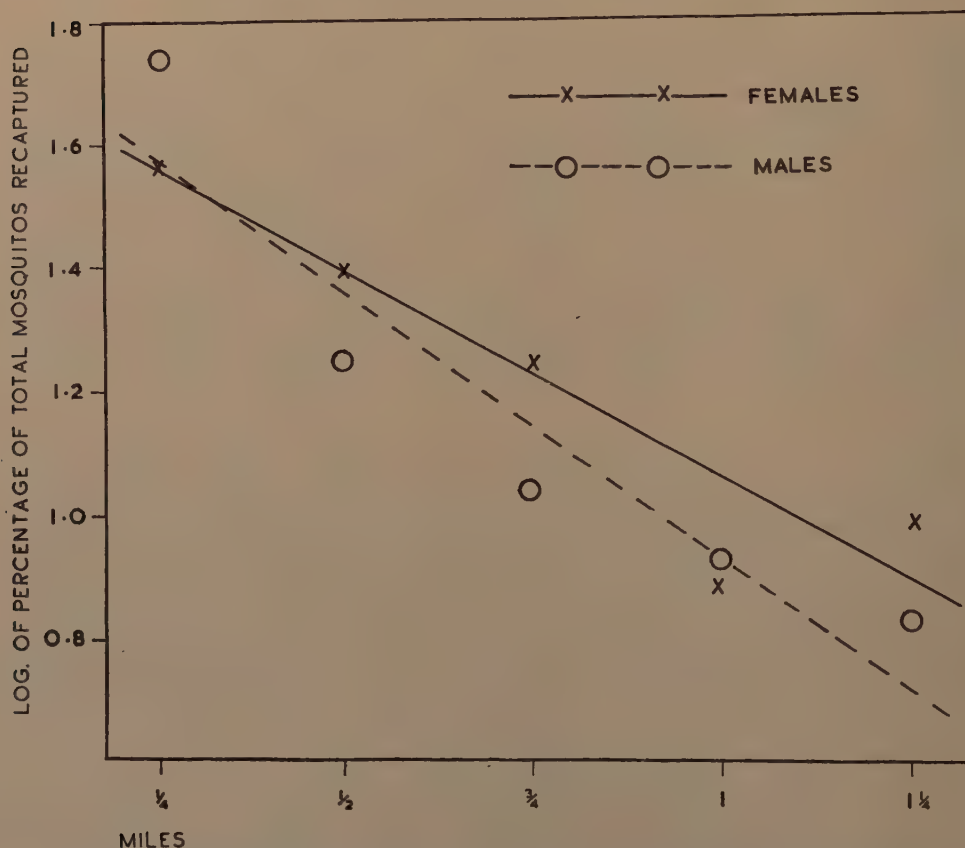


Fig. 3.—Regression lines of recaptures (expressed as log percentage of total recaptured) of isotope-labelled males and females in relation to distance (central releases).

more than two miles. On the same basis, the mean flight range of females is found to be 0.76 mile and of males 0.64 mile. However, recaptures were made at any point within each zone. Thus, the mean flight range is in reality $\frac{1}{3}$ th of a mile less than that calculated, and the figures given here should be—females, 0.64 mile, and males, 0.52 mile.

The recoveries from the peripheral releases, in which the recapture area extended in one direction up to a maximum of $2\frac{1}{2}$ miles, are shown in Table III. The left-hand section of the table shows the numbers recaptured, while the remaining part shows the estimated distribution at the different ranges. Details of the calculation of this estimate are shown in Table VII (all catches), which

takes into consideration the geometry of the recapture area (see figs. 1 and 2). The differences in the closer ranges between the painted and isotope-labelled females is largely due to differences in the frequency with which the various release points were used. In the isotope series the north-eastern point, which was situated in open country, was more frequently used than the south-western points, whereas the reverse was true for the painted releases.

TABLE III.

Recaptures of marked mosquitos from peripheral release points.

Range (miles)	Numbers recaptured			Estimated distribution*			Estimated distribution as percentages		
	Painted females	Isotope females	Isotope males	All females	Isotope females	Isotope males	All females	Isotope females	Isotope males
0- $\frac{1}{4}$	34	10	9	48	10	9	8	4	10
$\frac{1}{4}$ - $\frac{1}{2}$	60	4	5	75	6	8	12	2	9
$\frac{1}{2}$ - $\frac{3}{4}$	39	43	22	117	67	36	19	25	40
$\frac{3}{4}$ -1	15	13	3	64	30	7	11	11	8
1- $1\frac{1}{4}$	27	34	5	158	88	13	26	33	14
$1\frac{1}{4}$ - $1\frac{1}{2}$	13	10	1	68	30	3	11	11	3
$1\frac{1}{2}$ - $1\frac{3}{4}$	3	6	1	31	21	3	5	8	3
$1\frac{3}{4}$ -2	4	1	1	23	5	5	4	2	5
2- $2\frac{1}{2}$	1	2	1	20	7	7	3	3	8
Total	196	123	48	—	—	—	—	—	—

* See Table VII for method of calculation.

The pattern of dispersion from the peripheral releases is shown graphically in fig. 4. The most obvious features of this distribution are the relative scarcity of recaptures in the $\frac{1}{4}$ -mile zone, the somewhat uneven dispersion between $\frac{1}{2}$ and $1\frac{1}{2}$ miles, and the very small numbers captured beyond this range. This pattern is readily explained if the number of catching stations, and hence the density of human population, at the different ranges is taken into account. The break-downs

TABLE IV.

Estimated density of recaptures from peripheral release points, in relation to number of catching stations at different ranges (females only).

Range (miles)	North-eastern			South-western (I)			South-western (II)			Total per station	Per cent.
	Density	No. of stations	Density per station	Density	No. of stations	Density per station	Density	No. of stations	Density per station		
$\frac{1}{4}$	20	3	6.7	12	2	6.0	16	12	1.3	14.0	25
$\frac{1}{2}$	61	5	12.2	5	7	0.7	9	9	1.0	13.9	24
$\frac{3}{4}$	95	20	4.7	18	23	0.8	4	4	1.0	6.5	11
1	41	6.5	6.3	21	28	0.7	2	22	0.1	7.1	13
$1\frac{1}{4}$	143	26	5.5	7	18	0.4	8	32	0.2	6.1	11
$1\frac{1}{2}$	36	16	2.2	20	20	1.0	12	14	0.9	4.1	7
$1\frac{3}{4}$	24	15	1.6	7	12	0.6	—	9	—	2.2	4
2	23	12	1.9	—	10	—	—	13	—	1.9	3
$2\frac{1}{2}$	20	17	1.2	—	2	—	—	4	—	1.2	2

of estimated recaptures from the different release points, together with the numbers of catching stations at successive distances from them, are given in Table IV. Inspection of the table indicates the paucity of houses in the nearer zones, and their concentration in the middle zones, of the experimental area. In the table, the numbers recaptured per station have also been calculated, and in fig. 5 the logarithm of these values has been plotted against distance. This

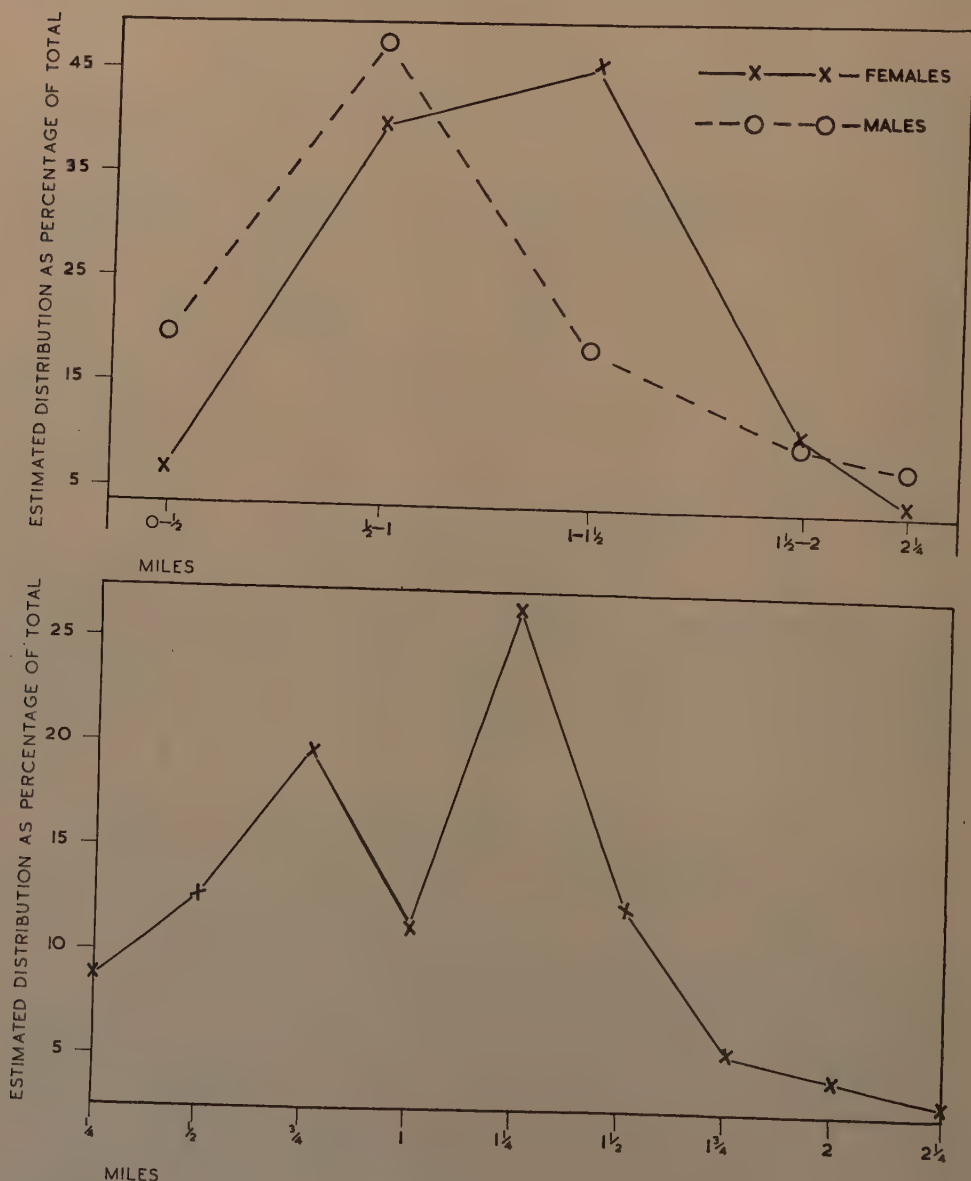


Fig. 4.—Estimated dispersion of marked mosquitos from peripheral release points, expressed as percentage of total recaptured. Above, isotope releases. Below, all females combined.

clearly shows a more or less straight-line distribution, the numbers recaptured falling off steeply with increasing distance. In other words, the irregular pattern of dispersion from these release points can be largely explained by the uneven distribution of villages within the experimental area.

An estimate of the flight range of the whole population released from the peripheral points is not easily obtained. But if the second part of the curve shown in fig. 4 is considered, in which the fall in density is more or less regular, and if the 1- to $1\frac{1}{4}$ -mile zone is taken as the starting point, the logarithm of the number of recaptures is found to decline at a rate of 0.23 per $\frac{1}{4}$ mile. If this regression is assumed to have continued beyond the limits of the experimental area, it can be calculated * that 50 per cent. of females flew less than one mile, 44 per cent. from one to two miles, and about 6 per cent. more than two miles. This gives a value of 1.1, or 0.98 miles if measured from the middle of each zone, as the mean flight range of the whole female population. The sample of males recaptured was too small for accurate estimation of flight range, but the upper part of fig. 4 shows very distinctly the more restricted range, although the pattern

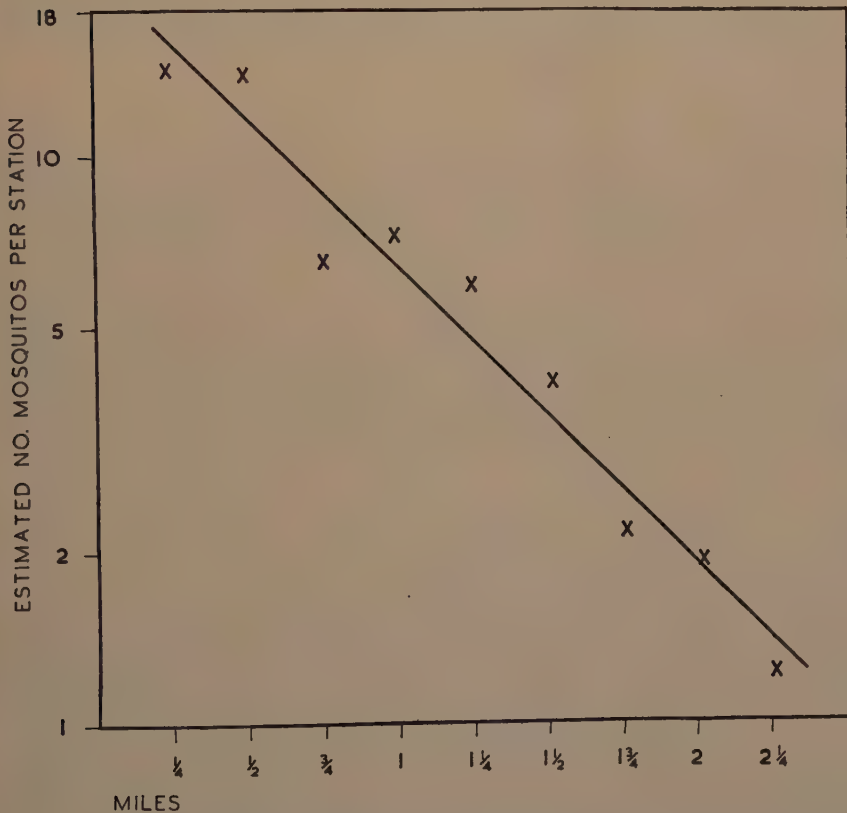


Fig. 5.—Density of recaptures per catching station in relation to distance.

* By giving the $1\frac{3}{4}$ -mile zone the value of antilog. 0.85, which is the mean value of the series of recaptures, expressed as percentages, from the $1\frac{1}{4}$ - to $2\frac{1}{4}$ -mile zones, and the $1\frac{1}{4}$ -mile zone the value of antilog. $0.85 + 2 \times 0.23 = 1.31$ or 20.4 per cent., and so on.

of dispersion was similar to the females. It should also be noted that individual males were recaptured at all ranges up to $2\frac{1}{4}$ miles.

To summarise these results, there is first of all an obvious relationship between flight range and density of human population. In the particular area chosen for the releases the dispersion of mosquitos from the centre, where the concentration of houses was high, was less than from the more sparsely populated peripheral areas. It also appears that, once the peripheral releases had spread into the centre of the area, their further dispersion was similar to those originally released in the centre. In other words, the somewhat greater flight range of the peripheral releases was not primarily due to the greater distance up to which recaptures were possible ($2\frac{1}{4}$ as opposed to $1\frac{1}{4}$ miles), but to the fact that they had further to go before coming under the influence of the central villages. It should also be noted that the distribution of breeding sites, as well as food sources, may have been significant. This could have been particularly important in the dry season, since the central area lay at a lower level than most of the outlying sectors where the surface water in the valley bottoms was less permanent.

These experiments have served to emphasise the limited meaning of the term 'flight range' as applied to a particular species. The actual movements of a mosquito population are clearly the product of the influence of topographical factors on its intrinsic flight characteristics, and the detailed results obtained here only refer to the flight range of *A. gambiae* in the type of country and with the density of human settlement described.

The effect of prevailing winds on dispersion.

Precise observations on the effect of wind on the movement of low-flying insects are always hindered by the presence of eddies and turbulence in the air stream, created by local irregularities in the terrain. In the present instance the situation was further complicated by the hilly nature of the experimental area. Consequently no attempt was made to measure the importance of local air currents on the flight of *A. gambiae*. On the other hand, the seasonal changes in the trade winds on the East African coast are regular and well defined, and it is possible to recognise two periods in the year, corresponding to the N-E. and S-E. monsoons, when the general wind direction remains constant for several months on end, with only short-term fluctuations in the vicinity of storms. An attempt was made, therefore, to assess the effect of this prevailing wind on the dispersion

TABLE V.

Mean flight range (in miles) of recaptures in relation to prevailing wind.

Release point	N-E. monsoon			S-E. monsoon		
	Males	Isotope females	All females	Males	Isotope females	All females
Central (northern sectors)	0.31	0.38	0.38	0.41	0.42	0.48
Central (southern sectors)	0.47	0.62	0.62	0.39	0.39	0.4
North-eastern (southern sectors only)	1.0	1.22	0.93	0.69	0.97	0.92

of mosquitos. In the experimental area the proximity of mountains had some influence on the movement of the air stream, as judged by the movement of low clouds, so that the locally effective direction of the S-E. monsoon, from late April to late October, fluctuated from West of South to a few degrees East of South. From mid-December to mid-March the wind blew steadily from the North-East. (It should be noted that the daily swing in direction, so noticeable on the coast itself, was of minor importance in the area studied.)

The average flight range of recaptured mosquitos in relation to the seasons as defined above is shown in Table V. Only the recaptures from the north-eastern and central release points are shown, since the south-western point was not in use all round the year. The results may be summarised as follows:

- (a) Of the mosquitos released in the centre of the area during the N-E. monsoon, dispersion against the wind (northern sectors) was appreciably less than in the southern sectors. The difference in the numbers flying over or under half a mile, to the south or north of the release point, is highly significant ($\chi^2=32.6$, $P=0.01$). On the other hand, this difference disappeared during the S-E. monsoon.
- (b) From the north-eastern release point, there was no seasonal change in the over-all flight range of female mosquitos, although, if the isotope females are taken alone, there is a small but significant difference ($\chi^2=5.43$, $P=0.02$) when the numbers flying under and over three-quarters of a mile are compared. Too few males were recaptured for further analysis.

These small differences are brought out more clearly if the recaptures are plotted diagrammatically, as in fig. 6. In particular, the numbers flying down-wind from the north-eastern release point into the extension area, during the S-E. monsoon, are clearly shown. The general impression given by these observations is that, in a populous and hilly region such as the experimental area, wind direction was a minor factor in aiding the dispersion of mosquitos.

TABLE VI.

Recaptures from central release point, showing dispersion in relation to age (females only).

Range (miles)	Day 1		Day 2		Days 3-9		Days 10+		All catches	
	Catch	Per cent.	Catch	Per cent.	Catch	Per cent.	Catch	Per cent.	Catch	Per cent.
$\frac{1}{4}$	28.5	59	27.8	61	54	29	18.7	27	129	37
$\frac{1}{2}$	10	21	6	13	53	28	15	22	84	24
$\frac{3}{4}$	5	10	4.5	10	35.5	19	16	23	61	18
1	1	2	6	13	26	14	13	19	46	13
$1\frac{1}{4}$	4	8	1	2	18	10	6	9	29	8
Mean range (miles)	0.45		0.45		0.62		0.65		0.58	

Dispersion in relation to age.

Up till now we have considered the flight range of marked mosquitos regardless of the time interval between release and recapture. It is important, however, to know what relationship exists between dispersion and age. For this purpose, four age-groups have been selected, representing those aged one day, two days, three to nine days and ten days and over, at the time of recapture. These groupings have been chosen since it was clear from the data that the movements of the

population during the first two days after release were generally different from those during the rest of the period over which mosquitos were recovered. The results are set out in Tables VI-VIII. It will be noted that in certain places the catches recorded are not whole numbers. As explained on p. 119, this results from the occasional use of the same mark on more than one day.

The distribution of recaptures up to $1\frac{1}{4}$ miles from the central release point for

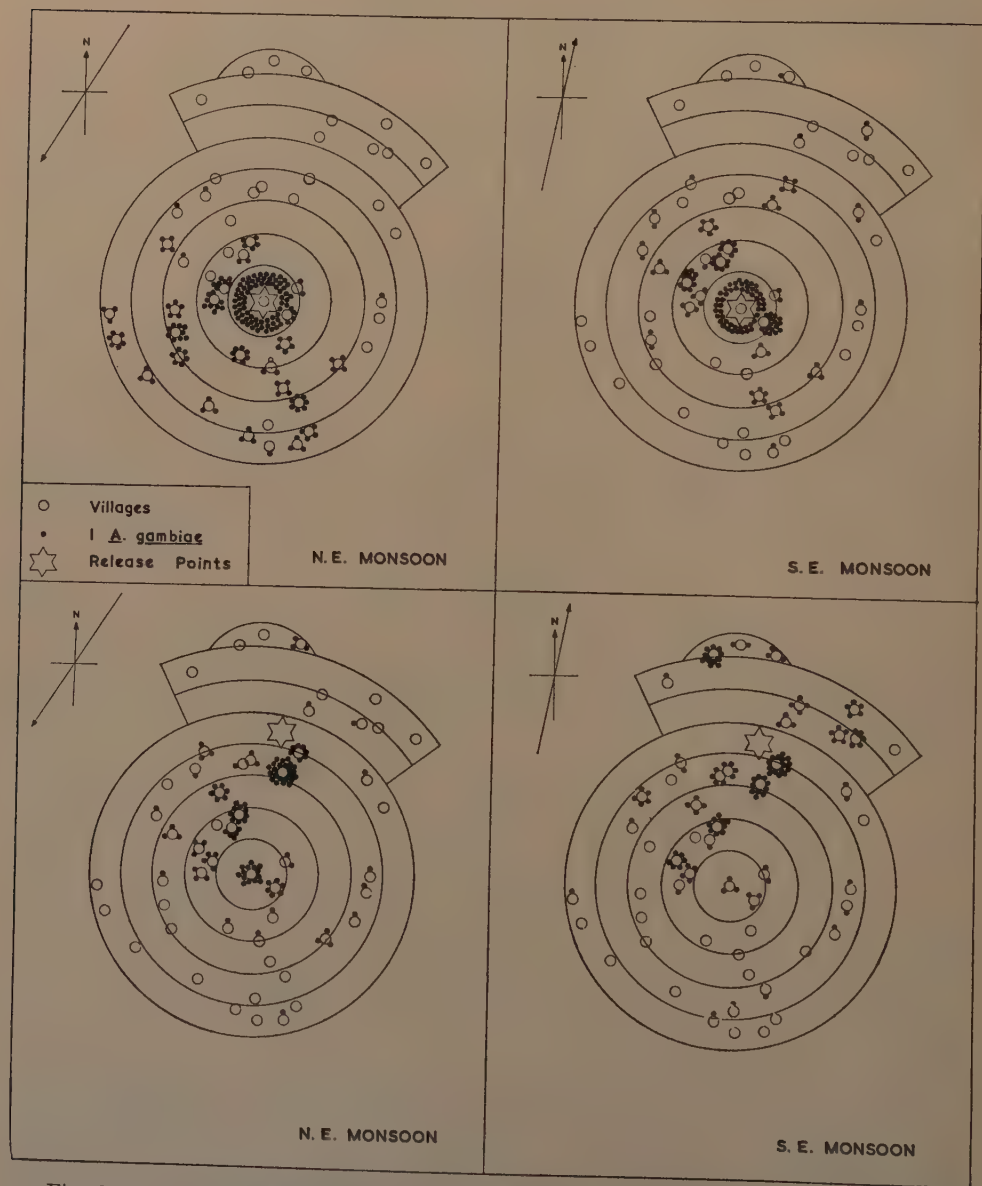


Fig. 6.—Dispersion of marked mosquitos in relation to prevailing wind. Each dot represents one individual of *A. gambiae* recaptured.

the four age-groups is illustrated in fig. 7. This shows very clearly that there are two phases of distribution, first, a period covering the initial two days after release, and secondly, the rest of the recorded life of marked mosquitos. In the first phase, marked females were mainly concentrated within a quarter of a mile of the release point, while in the second phase they were spread out throughout the

TABLE VII.

Recaptures and estimated dispersion from peripheral release points in relation to age (females only).

North-eastern release point

Range (miles)	Area factor	Day 1		Day 2		Days 3-9		Days 10+		All catches	
		Catch	Estimated density	Catch	Estimated density	Catch	Estimated density	Catch	Estimated density	Catch	Estimated density
$\frac{1}{4}$	1	—	—	1	1	12	12	7	7	20	20
$\frac{1}{2}$	1*	35	37	1	1	14.1	16.2	5.9	6.8	56	61
$\frac{3}{4}$	1*	7.3	8.3	13.1	15.5	31.6	47.2	17	24	69	95
1	2.4	3	7.2	2	4.8	8	19.2	4	9.6	17	40.8
$1\frac{1}{4}$	2.6	2.2	5.7	17.8	46.3	25	65	10	26	55	143
$1\frac{1}{2}$	3.0	1	3	—	—	10	30	1	3	12	36
$1\frac{3}{4}$	3.5	1	3.5	2	7	3	10.5	1	3.5	7	24.5
2	4.6	—	—	—	—	5	23	—	—	5	23
$2\frac{1}{2}$	6.7	1	6.7	1	6.7	—	—	1	6.7	3	20.1

South-western (I) release point

$\frac{1}{4}$	1	—	—	9	9	3	3	—	—	12	12
$\frac{1}{2}$	1.2	—	—	—	—	4	4.8	—	—	4	4.8
$\frac{3}{4}$	1.6	1	1.6	1	1.6	9	14.4	—	—	11	17.6
1	2.1	—	—	4	8.4	5	10.5	1	2.1	10	21
$1\frac{1}{4}$	2.4	—	—	—	—	1	2.4	2	4.8	3	7.2
$1\frac{1}{2}$	2.9	1	2.9	—	—	3	8.7	3	8.7	7	20.3
$1\frac{3}{4}$	3.5	—	—	—	—	1	3.5	1	3.5	2	7.0

South-western (II) release point

$\frac{1}{4}$	1.3	—	—	5	6.5	7	9.1	—	—	12	15.6
$\frac{1}{2}$	1.8	1	1.8	1	1.8	2	3.6	1	1.8	5	9
$\frac{3}{4}$	2	—	—	1	2	1	2	—	—	2	4
1	2.4	1	2.4	—	—	—	—	—	—	1	2.4
$1\frac{1}{4}$	2.6	—	—	1	2.6	1	2.6	1	2.6	3	7.8
$1\frac{1}{2}$	3	—	—	—	—	3	9	1	3	4	12

* Catches from the extension area have been multiplied by 2.

experimental area, but decreasing in density as its outer limits were approached. There is no significant difference in this pattern between the two older age-groups, a fact which suggests that, once the initial dispersion had taken place, mosquitos tended to be restricted to the same general area for feeding or oviposition. It also suggests that any continuing emigration of older mosquitos was at too low a level

to be detected. In this series of releases, however, recaptures were made only up to 1½ miles, and it is possible that the existence of a movement of this sort was masked by the limited area of recapture. Closer inspection is required, therefore, of the recoveries from the peripheral releases.

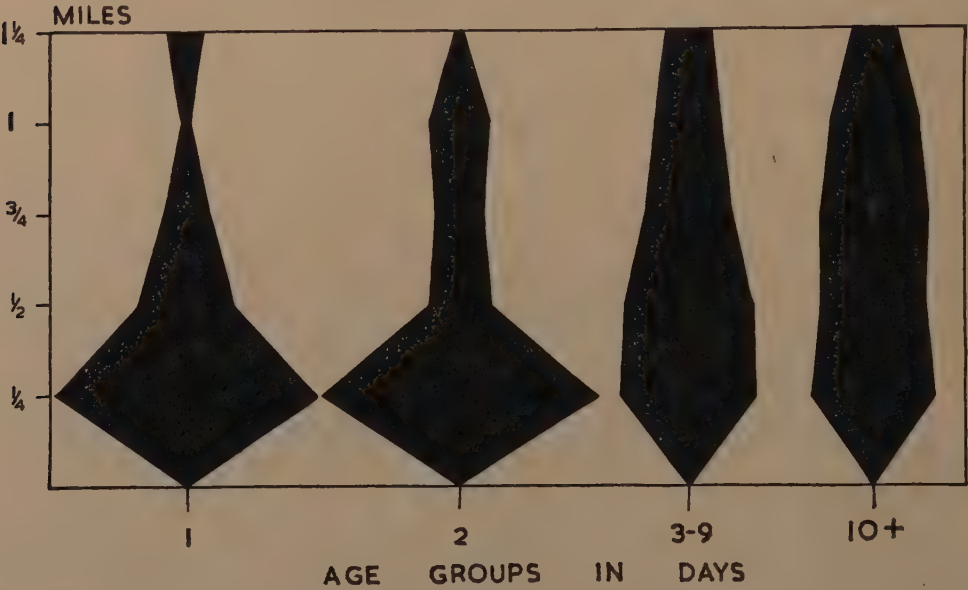


Fig. 7.—Dispersion from central release point by age-groups.

TABLE VIII.

Estimated dispersion of recaptures for all peripheral releases in relation to age (females only).

Range (miles)	Day 1		Day 2		Days 3-9		Days 10+		All catches	
	Density	Per cent.	Density	Per cent.	Density	Per cent.	Density	Per cent.	Density	Per cent.
1/4	—	—	16.5	14	24.1	8	7	6	47.6	8
1/2	38.8	48	2.8	2	24.6	8	8.6	8	74.8	12
3/4	9.9	12	19.1	17	63.6	21	24	21	116.6	19
1	9.6	12	13.2	12	29.7	10	11.7	10	64.2	11
1 1/4	5.7	7	48.9	43	70	24	33.4	30	158	26
1 1/2	5.9	7	—	—	47.7	16	14.7	13	68.3	11
1 3/4	3.5	4	7	6	14	5	7	6	31.5	5
2	—	—	—	—	23	8	—	—	23	4
2 1/4	6.7	8	6.7	6	—	—	6.7	6	20.1	3
Mean range (miles)	0.9		1.07		1.1		1.12		1.07	

The data in Table VII show the numbers recaptured and the estimated dispersion up to 2¼ miles for the same four age-groups. The estimated dispersion from all peripheral releases in relation to age, together with the mean flight range for each group, is shown in Table VIII. The same distribution is illustrated graphically in fig. 8. As was found for the central releases (fig. 7), both the two older

groups show an essentially unchanged pattern. Only in the recoveries from the south-western (I) release point is there any suggestion that the oldest group had an excess of females in the outer recapture stations; and here unfortunately the size of the sample was very small. With this exception, the findings from the recaptures of older mosquitos up to $2\frac{1}{4}$ miles parallel those up to $1\frac{1}{4}$ miles.

There is, however, one clear difference between the recoveries from the north-eastern release point and the central releases when the distribution on the second

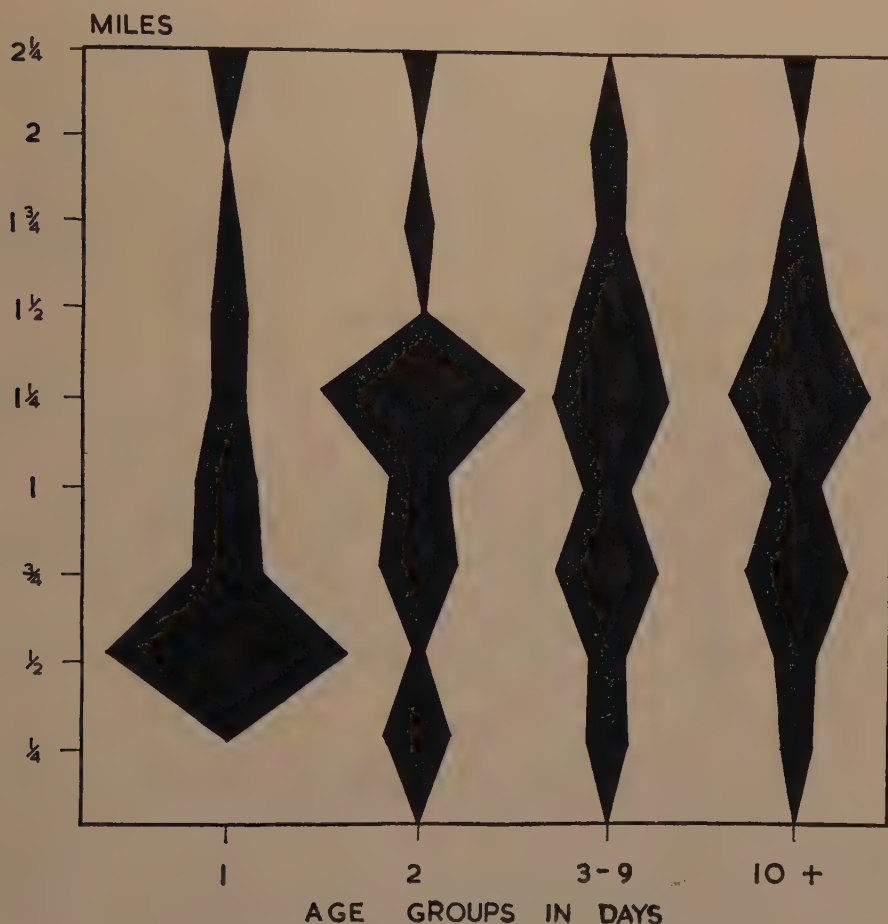


Fig. 8.—Dispersion from peripheral release points by age-groups.

day is considered. The dispersion of the latter group was virtually unchanged between the first and second days, whereas the mosquitos from the north-eastern point had already spread quite widely throughout the area by this time. The difference probably reflects the difference in density of houses and inhabitants in the two areas, although it was also influenced by the scarcity of catching stations in the vicinity of the peripheral point and consequent difficulty in ensuring adequate randomisation of catches on both days.

To recapitulate these findings, there is, first, a period of reduced dispersion lasting either one or two days. Its termination coincides approximately with the

first egg-laying flight. The second phase covers the rest of the observed life-span of marked mosquitos, and is characterised by an irregular distribution up to the limits of the recapture area. No change in the over-all distribution of the marked population between the earlier and later stages of this phase was detected, and no evidence was afforded therefore of a progressive dispersion with age. The existence of a continuing emigration from the experimental area on a small scale cannot, however, be excluded.

Age distribution of recaptures.

The distribution by age of all recaptures is set out in Table IX. In a number of releases the same label was used on two consecutive days or, more rarely, after an interval of one day. Consequently, mosquitos recaptured with such marks might have belonged to either day's releases. In setting out the results by age, such insects have been allocated fractionally to two different days. For example, in Table IX it will be seen that 0.4 males were recaptured on day 26 and 0.6 on day 28. Both refer to one specimen caught on August 20th, belonging to an unusually small rearing released in two batches of 500 and 300 on July 23rd and 25th, respectively. Since the chances are approximately 6:4 that the mosquito belonged to the earlier release, the recapture has been apportioned to the two days in the appropriate ratio.

A further correction has been applied to make allowance for the effect of interruptions of the recapture routine caused by weekends and holidays. The calculation is laborious and is not shown here. But one example will serve to explain the method. In the second series of isotope experiments there were eight releases, on day 2 after all eight, on day 3 after five, on day 4 after six, and so on. The method of working is shown in Table X. The third line of the table gives the number of recaptures per catching day, which provides the best value for the true age composition of the recaptured sample. However, there were several series of releases, each with different recapture rates. Since we want to combine all the series together again after correction, it is necessary to re-convert the corrected total of each series back to the original total, as shown in the lowest line of the table. The corrections have been made to all the series (see Table XI), and these figures are used in all further discussions on the age structure of recaptures.

TABLE X.

Method of correction for number of catching days; second series of isotope experiments (8 releases) (females only).

Age in days	1	2	3	4	5	6	7	8-23	Total
Total catch ..	35	45.8	20.2	17	9.2	25.8	14.6	65.4	233
No. of catching days (max.=8)	7	8	5	6	6	7	6	—	—
Mosquitos per catching day ..	5	5.72	4.04	2.83	1.53	3.69	2.43	9.7	34.94
ditto \times weighting factor ($233 \div 34.94 = 6.67$)	33.4	38.1	27	18.9	10.2	24.6	16.2	64.6	233

Detailed working is only shown for first 7 days after release. The weighting factor is applied so as to convert the total of corrected daily catches back to the original number recaptured.

TABLE XI.

Distribution by age of all mosquitos recaptured, after correction for number of catching days.

Age in days	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	
Painted	129.2	89.9	77.2	45.2	32.8	35.5	35.5	23.9	17.5	11.4	10.4	9.8	7	7.6	10	
Isotope ..	42.6	48.9	31.3	23.3	15.7	31	23.3	11.1	15.8	5.1	5.4	2.7	10.8	5.9	2.7	
Isotope ..	17.8	27.9	17.5	14.6	12.1	10.3	11	9.2	4.9	6.8	—	3.3	1.7	3.3	3.9	
All ..	171.8	138.8	108.5	68.5	48.5	66.5	58.8	35	33.3	16.5	15.8	12.5	17.8	13.5	12.7	
Age in days	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	Total
Painted	4.1	3.8	1.5	5.7	2	2.2	0.3	3.5	—	—	—	—	—	—	—	566
Isotope ..	4.7	0.9	3.3	1.1	5.1	2.4	2.1	1.8	—	—	—	—	—	—	—	297
Isotope ..	4.8	1.3	—	1.5	1.5	0.2	0.7	—	—	—	0.3	—	0.3	—	1.1	156
All ..	8.8	4.7	4.8	6.8	7.1	4.6	2.4	5.3	—	—	—	—	—	—	—	863

The general pattern of age distribution shown is one of a regular and moderately steep decline in numbers with increasing age. From Table XI it can be calculated that 77 per cent. of all females were caught during the first week after release (36% during the first two days), 17 per cent. during the second week, 6 per cent. during the third week, and just under 1 per cent. during the fourth week. No females older than 23 days were recaptured. The results of the two different marking methods are broadly similar. Regression coefficients for the logarithm of the numbers recaptured per two-day period (excluding days 1 and 2) are -0.09 and -0.067 for the paint and isotope releases, respectively. While suggestive, this difference is not significant at the 5 per cent. level ($t=1.9$). The two series of results have therefore been combined, and in fig. 9 the density of all recaptured females has been plotted on a semi-logarithmic scale in relation to age. The regression coefficient for this curve is -0.075 , which corresponds to a daily loss of mosquitos of 16 per cent.*

The age distribution of male mosquitos is also shown in Table XI. The regression coefficient for log density on age for males, for the period from 3-4 to 21-22 days, is -0.084 , which corresponds to a daily mortality of about 18 per cent. This is an unexpectedly low figure. It is commonly thought that males are short-lived insects. Yet their survival rate to 22 days is only slightly lower

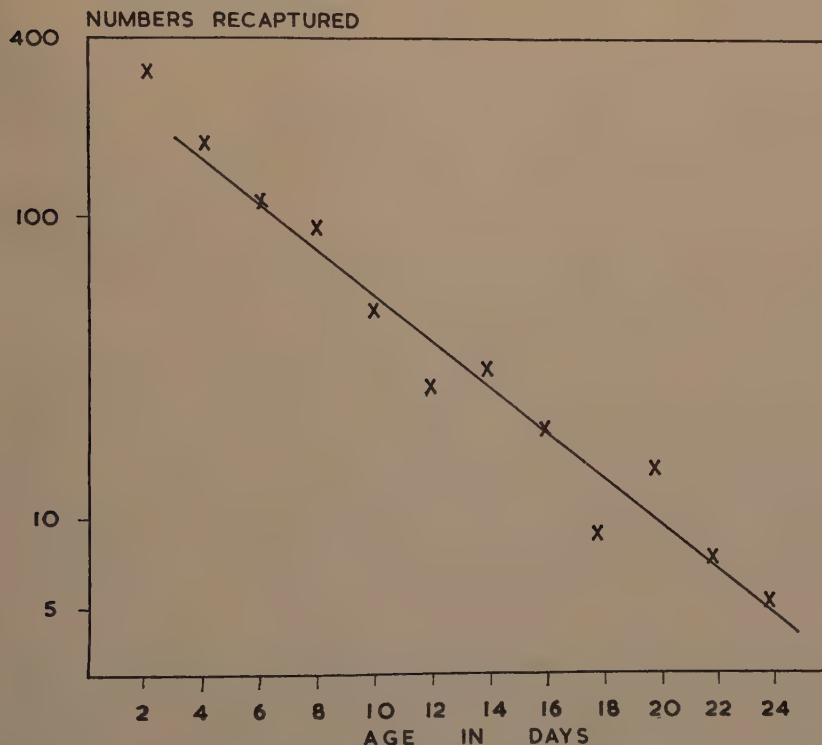


Fig. 9.—Numbers of recaptured mosquitos in relation to age.

* At age 3-4 days the expected catch is 159.6, and at 23-24 days it is 5; the decrease in 20 days is therefore 96.9 per cent., and 3.1 per cent. survive. Hence, if P is the chance of survival through one day, P^{20} , the chance of survival through 20 days, is 0.031, and $P = 0.841$. The chance of dying in one day is $1 - P = 0.159$, hence the percentage daily mortality is 15.9.

than that of the females, and, even more surprisingly, the oldest two mosquitos caught were both males, recaptured at 30 and 26 or 28 days after release.

Mortality rates of marked population.

As already pointed out, the slope of the curve in fig. 9 corresponds to the rate of loss of marked mosquitos from the experimental area. This loss may be the product of three factors, death, emigration, or failure of the marking technique. Evidence has already been adduced to suggest that the last factor was of negligible importance, and we are left, therefore, with the question of emigration. It is obvious from the spatial distribution of recoveries that a proportion of the insects was flying beyond the limits of the experimental area, even when released in the centre; and it is even more obvious that the peripheral releases must have resulted in an immediate substantial loss. The question to be answered is, how much did this loss continue to deplete the population, and to what extent did it vary with age? From fig. 7 it is clear that the first two days after release represented a phase of reduced emigration, and catches on these days should be excluded from the present discussion. But thereafter, although recaptures continued to be made up to the limits of the area, no change in their distribution could be detected. This indicates that the population had become stabilised after the first two or three days; and that, while there must have been a steady and continuing loss through emigration, this was at too low a level to have materially affected the distribution of mosquitos within the experimental area. That it was probably of minor importance is also suggested by the estimates of dispersion beyond the recapture area made in the section on dispersion in relation to age of recaptures. But it remains as one factor contributing to the rate of loss indicated by fig. 9. It cannot be assessed quantitatively from these data, and the 'mortality' rate of 16 per cent. derived from them refers to the combined loss through emigration as well as through death.

If the slope of the curve gives us only a limited amount of information, its shape, on the other hand, is of considerable interest. The densities of recaptures shown in fig. 9 have been plotted on a semi-log scale, and it will be seen that the points from day 4 to day 24 fall more or less on a straight line. This means that the rate of loss remained constant for all the age-groups shown; and unless, as seems highly improbable, the emigration rate fortuitously compensated exactly for any changes in survival rate, it must be concluded that the death-rate also remained stable throughout this period. It is possible that beyond this age the mortality rate may have risen, since recaptures of females ceased rather abruptly at 23 days. From the regression line shown in fig. 9 a total catch of some 8-10 mosquitos older than 24 days would have been expected. Their absence may have been due to a combination of the effects of chance together with a sharply reduced survival rate, or, less probably, to the simultaneous failure of persistence of both marking techniques. The implications of these tentative conclusions are discussed below.

Malaria infections in marked mosquitos.

Additional evidence on the survival of the population comes from the malaria infection rate among recaptured mosquitos. No malaria control measures were carried out in the experimental area, where the sporozoite rate in wild-caught *A. gambiae* averaged over all seasons 5-7 per cent. Since the chance of acquiring infection was the same for the marked as for the natural population, the sporozoite rate for the former should also be the same. However, of the limited number of gland dissections carried out, only three marked specimens positive for sporozoites were found. The results of the dissections are shown in Table XII.

Of those old enough to have mature sporozoites, that is, those aged 12 days

and over, only 3 out of 72.5 were positive. (For an explanation of the presence of fractions in these figures, see p. 119). A further 23 belonging to the same age-groups were not dissected, making an expected total of positives of 4 out of 95.5. In the same series of catches there were 390.5 mosquitos recaptured at an age below that at which malaria infection could be manifested. Hence the sporozoite rate of the whole population (aged three days and over) was 4 out of 486, or 0.8 ± 0.5 per cent. Recaptures aged one and two days have been omitted for the reasons given on p. 123. Had they been included, the infection rate would have been lower still. Thus, comparison of the infection rates in natural and marked mosquitos gives a further demonstration of the abnormally low survival rate of the released mosquitos within the experimental area.

Discussion.

The use of laboratory-reared mosquitos for marking and release experiments places an immediate limitation on the application of the results, particularly those relating to longevity. It may have some effect also on flight range, although, in view of the importance of the distribution of sources of food and oviposition sites in influencing the movements of the population, the use of reared mosquitos is unlikely to have affected the results to any very great extent. But as regards survival, it has already been pointed out that the rate at which females disappeared from catches amounted to 16 per cent. per day, the greatest part of this loss being attributed to mortality. Earlier work in the same district, Gillies (1958a) and Davidson & Draper (1953), had indicated that an average mortality rate of 7-8 per cent. per day would account for the age structure and malaria infection rate found in natural populations of *A. gambiae*. Thus, the figure obtained from marking and release experiments is too high by a factor of 2; and the most likely explanation of the discrepancy must be the condition of the mosquitos used. It would seem that this represents a major difficulty in any experiments to determine the longevity of *A. gambiae*, except perhaps in those places where larvae can be collected from natural waters in great numbers. However, comparison of the behaviour of artificially reared mosquitos, released in different areas and situations, may give valuable results, particularly in relation to dispersion.

When analysing the movements of insects it is important to distinguish between the decline in density with distance and the total numbers reaching any particular range. The density (numbers per unit area) will, of course, tend to fall owing to the increasing area over which they are spread. If a population is distributed at random, as pointed out by Russell & others (1944), the density will decrease regularly with distance, but the total numbers distributed at each range will be the same, provided a sufficient interval of time has elapsed. Under these conditions, the mean dispersion of the population will vary as the square root of the time (D. Yeo, The dispersal of insects—a theoretical model based upon random movement.—*Misc. Rep. colon. Pest. Res. Unit, Arusha* no. 249, 1959). It is doubtful whether many insects disperse from a localised focus in this manner, except over a very limited fraction of their total range, and the data obtained in these observations on *A. gambiae* confirm the conclusion of Russell & others (*op. cit.*), for *A. culicifacies* Giles, that dispersion is non-random. The alternative type of movement is that of flight within an ambit, in which the spread of the population is restricted by particular features of the environment.

It seems fairly clear from the present work that the movements of the marked mosquitos conformed to the latter pattern, the restricting features presumably being the concentrations of villages and breeding sites within the area. It would appear unlikely that the boundaries of such an ambit would be particularly sharply defined, as they may be for instance in the case of tsetse flies (Jackson, 1940), and a certain amount of exchange between adjacent localities must obviously occur. The size of the ambit will vary according to the nature of the terrain.

In open and sparsely inhabited country, mosquitos may range over a wide area with little in the way of recognisable limits. However, the subject has been little explored up till now, and it is not possible to describe the dispersion of tropical mosquitos with any degree of precision.

Some further discussion is needed on the question of mortality, the pattern of which, in the present experiments, led to the conclusion that the death-rate remained unchanged between the ages of 4 and 24 days. This implies that aging processes are unimportant in a wild population, and that the causes of death must be largely external, the result of accident or predation. As already remarked, however, it is possible that, beyond this age, physiological changes may have become important. The shape of the survivorship curve is of particular interest in relation to the epidemiology of malaria. If the mortality rate is constant, then as was demonstrated by Macdonald (1952, and later papers), the mathematical analysis of malaria transmission becomes greatly simplified. The point at present remains unsettled. Much of the evidence on it comes from studies on the survival of caged mosquitos, the results of which are somewhat conflicting and not wholly relevant. Field studies are scarce, and are mostly the work of Russian authors on *A. maculipennis* Mg. using advanced age-grouping techniques (see Gillies, 1958c). Of these, the most complete series is that of Detinova (1953), who found a steadily rising mortality rate among the older mosquitos. Her results, however, were not uniformly confirmed by workers in other parts of the Soviet Union. More recently, Zalutskaya (1959) has reported a study of the age condition of populations of *A. minimus* Theo. and *A. vagus* Dön. in North Vietnam. Closer examination of her results shows that, in both species, the mortality rate remained constant throughout the period of adult life for which detailed analysis can be made (age-groups "2-parous" to "6-parous"). In the case of *A. vagus*, of which a very large number of specimens were dissected, the fit is particularly close. Females that had laid more than six egg-batches, however, were less common than would be expected if the death-rate had remained the same. Thus, both Zalutskaya's work and the present findings with *A. gambiae* give some measure of support to the type of mortality pattern postulated by Macdonald. It suggests the possibility that, under tropical conditions, the importance of predation pressure may outweigh other factors in just the same way that the lethal effect of insecticides falls on young and old mosquitos alike. On the other hand, in cooler climates, they may be sufficiently long-lived for aging changes to become apparent.

Summary.

An account is given of marking and release experiments with *Anopheles gambiae* Giles in a coastal area of Tanganyika. Laboratory-reared mosquitos were used, labelled either by the topical application of paint or by the introduction of radioisotopes into the larval breeding pans. Two different isotopes were used, ^{32}P and ^{35}S , and recaptures were recognised by autoradiography.

Routine catching stations were established within a circle of radius of $1\frac{1}{4}$ miles. Releases were made either in the centre or near the periphery of the experimental area, so that recaptures were possible up to a maximum of $2\frac{1}{4}$ miles.

The following results were obtained:

1. Of 132,000 mosquitos released, 1,019 were recaptured.
2. The mean flight range of females released in the centre was estimated to be 0.64 mile, and of males 0.52 mile. Of females released on the periphery, the mean range of dispersion was estimated to be 0.98 mile. Individuals of both sexes were caught at the maximum range of $2\frac{1}{4}$ miles.

3. The dispersion of recaptured mosquitos was shown to be non-random and to be related primarily to the distribution of human settlements.
4. In certain series of releases the direction of the prevailing wind had a definite effect on the dispersal of mosquitos. But in general this was a minor factor.
5. Dispersion during the first day or, in many instances, during the first two days after release was more restricted, compared with that of older mosquitos. But no difference in the distribution of catches was detected between those aged three-nine days and those more than nine days old.
6. Marked females were recaptured up to 23 days after release. Apart from the first two days, the regression of density on age amounted to a daily loss of 16 per cent. of mosquitos from the experimental area. The effect of emigration could not be assessed quantitatively, but it was held to be a minor component of the total daily loss. The relatively high level of mortality suggested by these figures is attributed to the use of laboratory-reared mosquitos.
7. The corrected sporozoite rate in marked females at the time of recapture was 0.8 per cent.
8. The survival of males was only slightly lower than that of females.
9. It is concluded from the survivorship curve that the mortality rate remained constant throughout the period in which marked females were recovered.

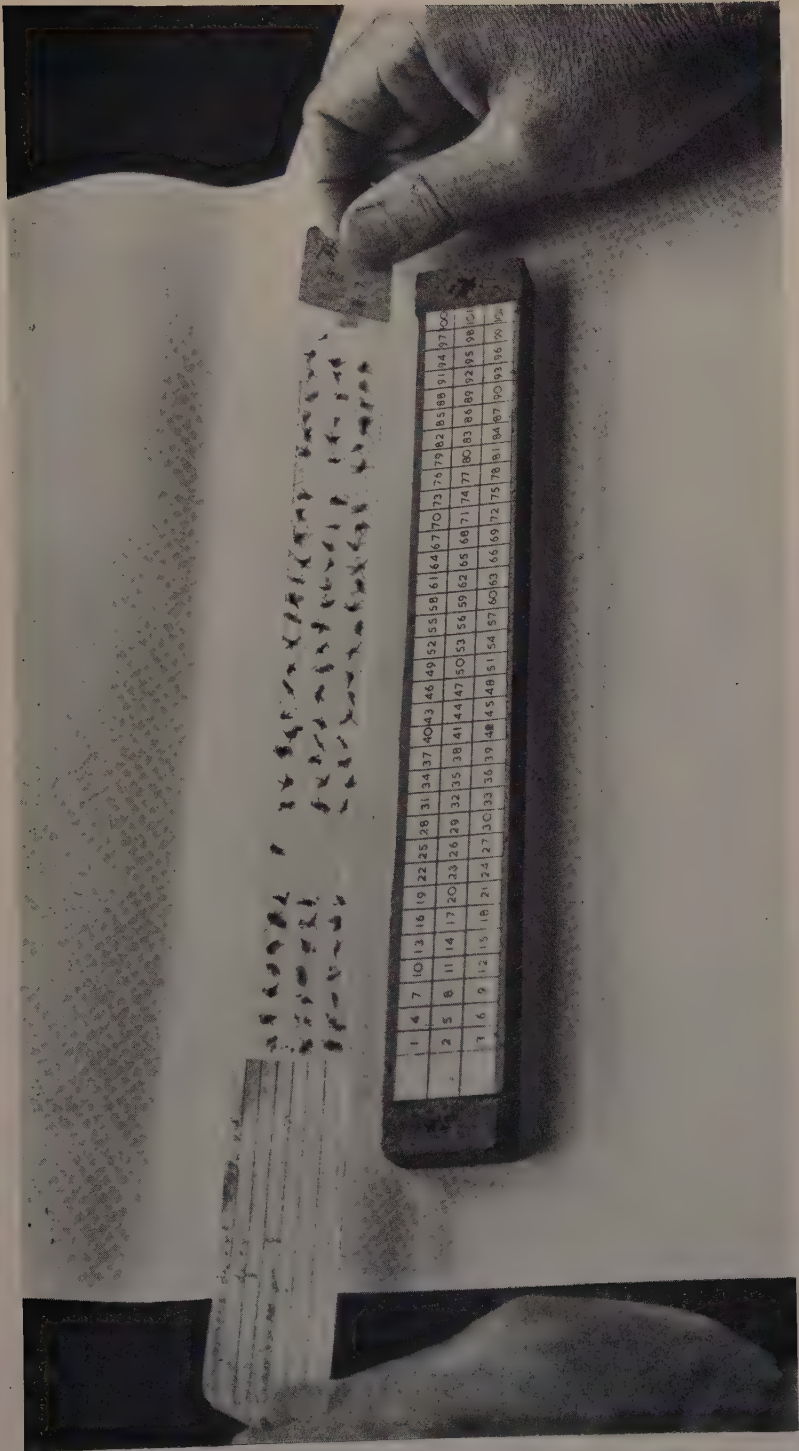
Acknowledgements.

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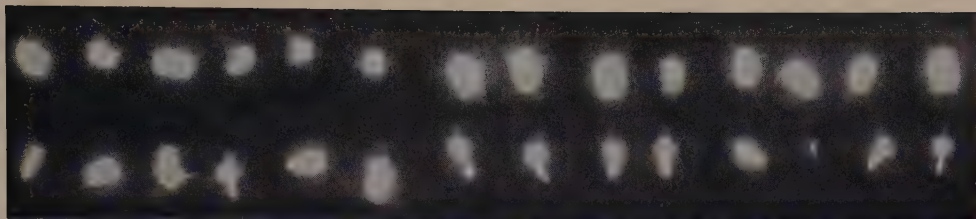
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Numbered grid used for checking origin of recaptured radioactive mosquitos.

FIG. 1. Newly emerged mosquitos labelled with ^{32}P .FIG. 2. Newly emerged mosquitos labelled with ^{35}S .

FIGS. 3-7 (left to right). 3. A male, labelled with ^{35}S , recaptured after 30 days. 4. A female, labelled with ^{35}S , recaptured after 19-21 days. 5. A female, labelled with ^{35}S , recaptured after 18 days. 6. A male, labelled with ^{32}P , recaptured after 21-22 days. 7. A female, labelled with ^{32}P , recaptured after 20 days.

THE SORGHUM MIDGE, *CONTARINIA SORGHICOLA* (COQ.), IN NIGERIA.

By K. M. HARRIS

E.M.N.

*Entomologist, Department of Agricultural Research,
Ibadan, Nigeria.*

(PLATE III.)

Barnes (1954a, b) drew attention to the widespread occurrence of the sorghum midge, *Contarinia sorghicola* (Coq.), in countries where sorghum is cultivated and stressed the need for further studies of this pest and of related Cecidomyiids which might cause considerable losses of grain in tropical countries where sorghum is the staple cereal.

C. sorghicola has probably been present in Nigeria for many years but the absence of grain in spikelets which have been attacked by it (Pl. III, fig. 1) has generally been attributed to genetic sterility or unfavourable growing conditions and only recently has the midge been recognised as a pest of Nigerian cultivated sorghums.

It was first recorded from Ibadan, Western Nigeria, in March 1953 and, later in the same year, in a preliminary survey of the distribution of gall midges attacking the seed heads of grain sorghums and wild grasses, *C. sorghicola* was obtained from a number of widely separated localities (Sutherland, 1955).

This survey was followed by the studies of the present author which have been centred at the Regional Research Station, Samaru, Zaria, and have been mainly confined to the Northern Region in which cultivated sorghums are of great importance.

Host range and distribution.

The main hosts of *C. sorghicola* in Nigeria are the cultivated varieties of the genus *Sorghum*. Snowden (1936) classified the cultivated sorghums under 31 species within this genus but the validity of such a classification is now doubted and there is an increasing tendency to consider the many cultivars as members of a single extensive species, *S. vulgare* (Quinby & Martin, 1954).

In Nigeria, *C. sorghicola* has been reared from a wide range of varieties representing all of the six sub-series of cultivated sorghums which Snowden defined (*op. cit.*) and has also been reared on many occasions from the wild grasses *Sorghum arundinaceum* and *Andropogon gayanus*. Barnes (1956) recorded that midges which were apparently indistinguishable from *C. sorghicola* had been reared from *Panicum maximum* in Nigeria and from *Pennisetum polystachyon* in the Gold Coast, but these records have not been confirmed in subsequent collections of midges reared from these two grasses in Nigeria.

During the initial survey of the food-plant range of *C. sorghicola* in Nigeria, Sutherland obtained three midges other than *C. sorghicola* from seed heads of the cultivated sorghums. Two of these, an undescribed species of the genus *Lestodiplosis* and a *Clinodiplosis* midge which is known only from its larva, do not attack the grain. The third, a species of *Stenodiplosis*, was considered to be a potential grain pest along with a species of *Lasiopterariae* reared from wild sorghum, *S. arundinaceum* (Barnes, 1954a; Sutherland, 1955). It now appears that both of these midges are of very little importance in Northern Nigeria and are

only found regularly in Western Nigeria outside the area of intensive sorghum cultivation and that in both regions *C. sorghicola* is by far the most important gall midge attacking the grain of sorghum.

There are no definite records of *C. sorghicola* from Eastern Nigeria, though there is little doubt that it occurs there.

The guineacorn crop in Nigeria.

A wide range of sorghum varieties is grown in Nigeria and the crop is known as guineacorn. The annual production of guineacorn in the Northern Region is about two million tons of grain, which is used almost entirely for human consumption and provides the staple food of most of the population.

The crop is sown early in the rains and most varieties mature in 5 to 7 months with earlier-maturing varieties predominating in higher latitudes where the growing season is limited by the longer dry season. In most areas one particular variety is preferred and long-stalked varieties are generally favoured since the stalks may be used for building and fencing.

Heads are formed when the stems are over 10 ft. high and flowering commences as soon as the head has emerged from the boot-leaf. Anthers first appear on the spikelets at the tip of the head, and during the next five or six days a wave of anthesis spreads down the head. Each hermaphrodite spikelet produces a single grain which is at first enclosed by the glumes. The grain ripens about six weeks after anthesis and is harvested on the head and threshed out as required.

Some farmers tend the crop well, but all too often insufficient attention is given to thinning and weeding, or development is hindered by the practice of inter-sowing guineacorn in early millet, in which case the guineacorn is over-shadowed during its early growth. Yields are considered good if they exceed 1,000 lb. grain per acre.

Life-history.

The life-history of *C. sorghicola* on guineacorn has been studied in detail at Samaru.

From July until November, the daily emergence of adults from pupae in infested heads begins shortly after daybreak (6.15–6.45 a.m.) and reaches a peak between 7.45 and 8.15 a.m. The males take flight a few minutes after emergence and fly above the head from which they have emerged. They alight to copulate with the emerging females and then resume their flight. In the field, females usually mate immediately after they have emerged from the pupa and during mating and for up to an hour after mating they remain on the head from which they emerged. They then fly off in search of guineacorn heads which are in a suitable condition for oviposition.

The female chooses for oviposition heads which have recently flowered and, having found a suitable head, begins to lay. During the whole period of egg-laying she maintains a continuous agitated exploration of the spikelets with her long extensile ovipositor. The ovipositor is inserted between the glumes, which have been slightly parted by the emergence of the anthers during anthesis, and eggs are laid within the spikelet. While depositing an egg the exploratory movements cease and the midge remains motionless on the selected spikelet. If watched closely the convulsive passage of the egg down the oviduct can be detected, and following this the female immediately resumes her agitated exploratory movements. From time to time the midge makes short flights which, if the head on which she originally alighted is suitable for oviposition, are confined to flights within the head. The tenacity with which the female may hold to a favourable head is quite remarkable: on one occasion two females remained on the same head for more than three hours despite a very boisterous wind.

During the main flowering period of the crop, ovipositing females are most abundant between 9.0 and 11.0 a.m. Peak activity is reached at different times on different days but the maximum usually occurs between 10.15 and 10.45 a.m.

In the laboratory, mated female midges oviposit readily when caged on guinea-corn heads or when confined in petri dishes on sprigs of guineacorn spikelets. Under these conditions the average number of eggs laid by 52 females ovipositing for 3 to 5½ hours was 25 per female with a range from 7 to 37. The maximum rate of oviposition observed was the deposition of five eggs in seven minutes by a single female. Dissections of females which have not oviposited show that the ovaries contain an average of just over 50 eggs with a range of 25 to 90 and this is taken as a measure of the potential oviposition under natural conditions. In the local main-crop variety Farafara, most eggs are deposited just inside the tip of the spikelet and twice as many eggs are laid on the glumes as on the pales.

The female occasionally lives longer than 24 hours, but more usually dies within ten hours of emergence. The male is shorter-lived and is usually dead before 2 p.m. on the day of emergence.

The egg hatches within four days, and from the fourth day onwards larvae are present under the pales lying close against the ovary. Here they remain and develop at the expense of the ovary which shrivels up and fails to develop, and as a result the spikelets that have been attacked have a flattened, empty appearance (Pl. III, fig. 1). Many eggs may be laid on one spikelet, but usually only one larva develops to maturity, though cases of two and even three pupae forming in one spikelet are not uncommon.

After feeding for ten days the larvae pupate, and at first the pupa lies against the ovary in the position originally occupied by the larva. Shortly before the adult is due to emerge, the pupa moves up to the tip of the spikelet and at emergence it protrudes from the spikelet.

The life-cycle from egg to adult is completed in 19 to 22 days during the growing season, but towards the end of the rains an increasing number of larvae fail to pupate. These spin a cocoon and remain in diapause within the spikelets until the diapause is terminated in subsequent growing seasons.

Ecology.

Climate is of primary importance in limiting populations of *C. sorghicola*. In the south-eastern United States, breeding ceases in the fall and the midges hibernate as cocooned larvae from which adults emerge in the following spring between 10 and 20 days after a fall of rain (Walter, 1941), and in Queensland the midge becomes active two weeks after wet weather and high humidity (Passlow, 1954). Cowland (1936) observed that, in the Anglo-Egyptian Sudan, adults from the resting generation emerge in the latter part of the rains, and Geering (1953) reported that at Namulonge in Uganda, midge attack was continuous on a succession of sowings of sorghum, and diapause larvae, which appeared in December, February, March and April, all produced adults in August after a 20-day period of low saturation deficit of the air.

Dean (1911) recognised the importance of a prolonged flowering period in producing high midge populations in the United States and more recently seasonal increases in population through early- to late-flowering varieties have been noted in the Sudan (Anon., 1953) and in the Gold Coast (Bowden & Neve, 1953).

Barnes (1956) comments on the danger of accumulation of cocooned larvae and also reviews the known parasites and predators which appear to be of little importance in limiting the initial increase in population.

Seasonal biology.

At Samaru, fluctuations in midge activity were studied during the period 1955 to 1959. The severe dry season of at least four rainless months begins in October

and as soon as rainfall ceases the atmospheric relative humidity decreases rapidly. The midge continues to breed during November but, as humidity and temperature fall, an increasing proportion of larvae enters diapause. Temporary resurgences of higher humidity may prolong the breeding period but before the end of November emergence of adults ceases.

Most of the diapause larvae survive the dry season in those heads of the main crop which flower late in the season and are severely attacked by the midge. Because they contain little or no grain they are not harvested but remain on the stalks which, after harvest, are gathered into large stacks and kept through the dry season to be used to build huts and fences or to be burnt as fuel. Samples of 50 spikelets taken at random from such heads contained up to 83 diapause larvae with a mean over the four years of 23 larvae per sample. Each head contains at least 300 spikelets and, in a medium-sized village near Samaru, 5,000 such heads may be collected with ease. Some diapause larvae are present in threshing trash and also on harvested heads which are stored through the dry season, but both at Samaru and throughout most of the major guineacorn-growing area, which lies between latitudes 10 and 13°N., it is the heavily infested unharvested heads which are mainly responsible for the carry-over of the midge from one season to the next.

Once the midge larvae have entered diapause there is little mortality, and the magnitude of the diapause population is largely a reflection of intensity of midge attack at the end of the season, though the fact that some adults have emerged from larvae up to two years after the beginning of diapause indicates that there may be an accumulation of diapause larvae over a number of seasons.

The larval diapause continues through the dry season, and the first adults emerge half-way through the rainy season when atmospheric relative humidity is rising and temperature is falling. The emergence of adults from diapause larvae has been observed under experimental conditions. At the beginning of each dry season, sorghum heads containing diapause larvae were placed in cages and exposed to the weather. The cages were examined daily, and any adult midges were collected and identified by examination under a microscope. Under these conditions the emergence of adults of *C. sorghicola* commenced between 5 and 7 weeks after the weekly mean R.H. had risen above 60 per cent. and emergence continued for 9–12 weeks. The appearance of the first adult midges on flowering sorghum on the research station and local farms was also recorded and the experimental and field observations are compared in Table I.

TABLE I.

Dates of first records of adult midges in cages and in the field.

		1956	1957	1958	1959
Cages	..	5th July	22nd June	24th July	9th July
Field	..	6th July	27th July	1st Aug.	22nd Oct.

In 1956, there was close agreement between the two observations but an increasing discrepancy appears in subsequent years. This is attributed to the fact that, on the research station, improved control of the midge by destruction of crop residues, and also, in 1959, by the use of insecticide, has reduced the carry-over population to a low level so that detection of the first midges in the field has become increasingly difficult. The emergence of midges in the cages is considered to give a fairly accurate impression of the pattern of emergence under natural

conditions, and it is concluded that, at Samaru, *C. sorghicola* breeds only during the period June–November, when the climate is favourable.

The data obtained in 1958 and 1959 are summarised in fig. 1 and it may be seen how the emergence of the midge from diapause precedes the flowering of the main guineacorn crop. Since adult females live but one day, they must find flowering guineacorn shortly after emergence or die without ovipositing, and the availability of flowering guineacorn during this period preceding the main-crop flowering is therefore of considerable importance in controlling the seasonal build-up of the midge population. So long as sufficient guineacorn is in flower and the weather is favourable the midge population builds up steadily and reaches its highest level in late October and early November.

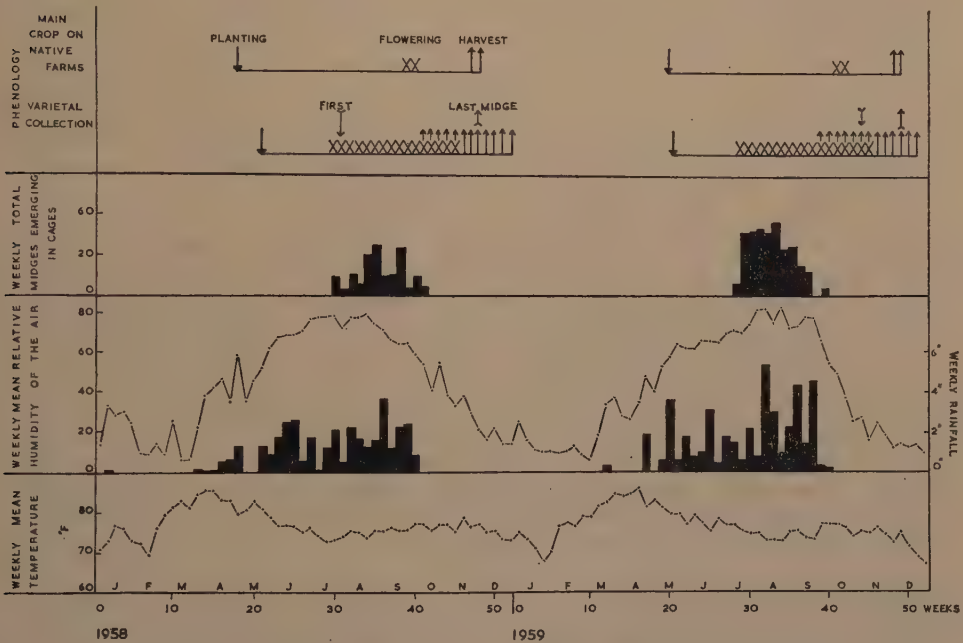


Fig. 1.—Factors affecting the seasonal biology of sorghum midge at Samaru.

In native farms at Samaru there is little or no guineacorn in flower during most of the period when adult midges are emerging from diapause and, despite the presence of large numbers of diapause larvae which have survived the dry season in crop residues, the initial increase of the breeding population is slow. In contrast, the plant-breeder's varietal collection on the research station at present contains flowering guineacorn throughout this period and the uninterrupted breeding of the midge results in far more severe damage in the later-flowering varieties than has ever been seen on the main crop in local farms.

Wild-grass food-plants.

The known wild food-plants of *C. sorghicola* in Nigeria are *Sorghum arundinaceum* and *Andropogon gayanus*.

A. gayanus is abundant throughout Northern Nigeria and is often the dominant grass around cultivated land. It could, therefore, be a most important alternative food-plant of sorghum midge but, although there are no morphological differences

between *C. sorghicola* from *S. vulgare* and from *A. gayanus*, there are biological differences which indicate that a distinct race of *C. sorghicola* occurs on *A. gayanus*.

In a series of experiments in which mated females of *C. sorghicola* were transferred from one food-plant to the other, 112 females from *S. vulgare* failed to lay eggs on flowering heads of *A. gayanus*, and 20 females from *A. gayanus* refused to oviposit on *S. vulgare*. Occasionally females became excited when first caged on heads but after a few short preliminary attempts at ovipositing no further attempts were made. This behaviour is in marked contrast to the behaviour of mated females from *S. vulgare* caged on *S. vulgare*, which began to oviposit immediately and continued for up to 5½ hours.

The readiness with which virgin females from *S. vulgare* and from *A. gayanus* mate with males from the other food-plant was tested by confining pairs of newly emerged midges in 1×3 in. specimen tubes and watching them for up to 30 minutes. In 21 attempts to mate virgin females from *A. gayanus* with males from *S. vulgare*, 6 pairs copulated but only one female oviposited when caged on *A. gayanus*, and, in 10 attempted matings between virgin females from *S. vulgare* and males from *A. gayanus*, 2 pairs copulated and one female oviposited on *S. vulgare*. In both cases where eggs were laid they failed to hatch. Again the behaviour of the midges was in marked contrast to the behaviour of virgin females from *S. vulgare* confined with males from the same food-plant; under these conditions, they invariably mate immediately.

Further experiments must be made before any definite conclusions on the status of *C. sorghicola* on *A. gayanus* may be drawn, but this preliminary evidence, together with field observations made during the past two years, strongly suggests that *A. gayanus* is not an alternative food-plant of *C. sorghicola* on guineacorn.

Biological testing of *S. arundinaceum* has not been attempted but its very close affinity to the cultivated members of the same genus gives little cause to doubt that the midges which have been reared from it are biologically as well as morphologically identical with *C. sorghicola* on guineacorn. Its importance as an alternative food-plant in Northern Nigeria is limited, since it is largely confined to river banks and is frequently found in the main guineacorn-growing areas, but further south, where it is more abundant and more widely distributed, it may be of some importance as an alternative food-plant.

Parasites and predators.

At Samaru, the following parasites have been reared from heads infested by *C. sorghicola*:

Eupelmus popa Gir.

Eupelmus sp. 'popa group'—previously reared from a Lasiopterarian midge in *Brachiaria* in Uganda.

Aprostocetus sp.

Tetrastichus sp.—previously reared from sorghum midge by Geering in Tanganyika, 1953.

Tetrastichus sp.

All three genera have also been recorded from a number of widely separated localities both in Northern Nigeria and in Western Nigeria.

At Samaru, the parasite population is most evident late in the season when the midge population is at its peak. *Eupelmus* is generally most abundant, and examples of the three genera tend to be present in the ratio 3 of *Eupelmus*:1 of *Aprostocetus*:1 of *Tetrastichus*. They exert little control over the midge before the main crop flowers, but play an important part in controlling the population

later in the season, particularly when the climate favours the midge and breeding continues up to late November.

Two species of spider, referred to as *Thomisus* and, tentatively, *Diaea*, prey on ovipositing female midges but appear to be of minor importance.

Crop losses.

The presence of a single midge larva developing within a spikelet is sufficient to prevent the formation of the grain and, since this loss of grain occurs at a critical period in the development of the crop, the loss in yield should be directly related to the proportion of spikelets attacked. Experiments have shown that this is so and that there is a significant negative linear regression of yield on the proportion of spikelets attacked.

In the early experiments, midge damage was assessed visually but though this method produced some information the proportion of spikelets attacked was underestimated. (Table II.)

TABLE II.

Regression analysis of yield on the proportion of spikelets attacked by sorghum midge.

Locality and year	Range of midge damage (% spikelets attacked)	Regression equation
Samaru, 1955	0-26%	$Y = 7.44 - 0.533(x - 13.71)$ lb. grain per $\frac{1}{16}$ th acre. $t = 3.13^*$ d.f. 4
Kafinsoli, 1955	4-38%	$Y = 14.59 - 0.317(x - 15.80)$ lb. grain per $\frac{1}{16}$ th acre. $t = 2.78^*$ d.f. 8
Kano, 1955 ..	0-36%	$Y = 25.20 - 0.417(x - 14.10)$ lb. grain per $\frac{1}{16}$ th acre. $t = 3.07^{***}$ d.f. 18
Samaru, 1956	0-19%	$Y = 0.460 - 0.0135(x - 4.7)$ lb. grain per 5 heads. $t = 3.80^{***}$ d.f. 39

In 1958, the estimation was based on dissection of random samples of spikelets, but midge attack failed to develop and it was not until 1959 that an accurate estimate of the regression equation was obtained by classifying and counting all spikelets and weighing all grain from a sample of heads taken from one row of an acre of main-crop guineacorn at Samaru. The results are summarised in fig. 2.

The possibility that some compensation for midge attack might result from the production of larger grains in the unaffected spikelets was also investigated in this experiment. The weight of 100 grains in each head was compared with the proportion of spikelets attacked and no evidence of any relationship between the two observations was found. It is concluded that estimation of the proportion of spikelets attacked by midge gives a direct measure of the loss of yield.

Before 1957, no estimate of the extent of grain loss in native farms had been made. It was known that on the experimental farms of the Department of Agriculture losses were often great but, for reasons already discussed, such losses were considered to be atypical. A survey was therefore designed on the basis of the studies of *C. sorghicola* which had been made at Samaru.

It was known that the midge oviposits on guineacorn shortly after flowering and that development to the adult takes 19 to 22 days. Midge larvae had been easily detected in samples of spikelets dissected seven days after midges had oviposited, and field officers throughout Northern Nigeria were therefore asked to

collect samples of guineacorn spikelets between 7 and 21 days after 50 per cent. of the crop had flowered.

In defining the sampling areas, local problems of staffing and communications had to be considered and the final selection was left to the discretion of provincial staff with the proviso that all samples should be taken from native farms within the main guineacorn-growing areas of the province and that these farms should be at least five miles away from any experimental farm.

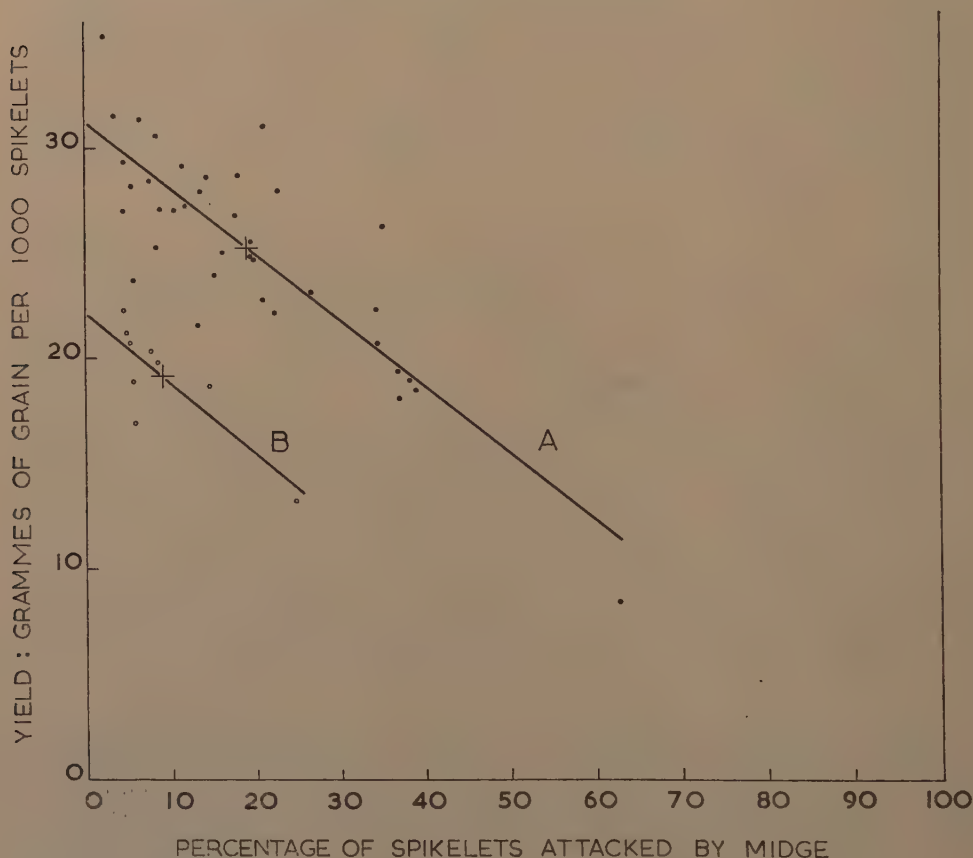


Fig. 2.—The effect on the yield of guineacorn of attack by sorghum midge.

A: less than 10 per cent. spikelets attacked by agents other than midge.

$b_A = -0.316$ $t = 9.6***$

B: 31-40 per cent. spikelets attacked by agents other than midge.

$b_B = 0.329$ $t = 3.8**$

Each area was sampled by walking along a single row in as many guineacorn fields as possible and removing a sprig of spikelets from every tenth head. Sprigs were taken successively from the top, middle and bottom of the head to make allowance for any variation in the midge infestation within heads and all sprigs collected in a sampling area were sealed in a cellophane bag with a label which gave details of the locality, date of sampling, varieties sampled and number of villages and fields included in the sample. The bags were then sent to the Regional Research Station, Samaru, where the assessments of midge damage were made.

On arrival at Samaru, any adult midges which had emerged in transit were collected, counted and preserved and the samples were then dried in the sun and stored to await dissection. The selection and dissection of sub-samples continued from October to February and since humidity is very low at this time of year the samples awaiting dissection remained in excellent condition and no midges emerged from them.

A sub-sample of 50 spikelets was taken at random from each sample and dissected under a binocular microscope. Eggs and first-instar larvae of the midge could not be detected with certainty but all other stages were easily found and the number of spikelets containing midge was recorded for each sub-sample. In addition, spikelets which had not flowered were distinguished by the presence of anthers within the glumes and were also counted. The accuracy of this method was checked by selecting ten separate sub-samples from each of five survey samples representing various levels of midge attack and was found to be satisfactory. (Table III.)

TABLE III.

Results of dissection of ten independent sub-samples from each of five survey samples.

Sub-sample no.:	1	2	3	4	5	6	7	8	9	10
Sample 495 ..	0	0	0	0	0	0	0	0	0	0
„ 32 ..	0	3	0	1	1	0	0	0	1	0
„ 205 ..	0	2	2	2	1	4	2	2	0	5
„ 31 ..	8	6	10	9	7	7	8	9	7	6
„ 489 ..	22	29	35	28	36	28	20	25	22	23

The survey covered all provinces of Northern Nigeria and a summary of the information obtained is given in Table IV.

In 1958, more samples were collected because the 1957 survey had aroused interest and fewer villages and fields were included in a sample because collection of a sample was restricted to a single day and not spread over a number of days

TABLE IV.

Sorghum-midge survey, Northern Nigeria: Summary.

	1957	1958
Number of samples	163	307
Average number of villages and fields included in a sample	4.5 villages, 26.5 fields	3 villages, 13 fields
Number of midges emerging in transit	335	208
Number of spikelets dissected	8150	15350
Number of spikelets containing midge	357 (4.4%)	489 (3.2%)
Number of midge larvae and pupae found in spikelets ..	525	800
Number of spikelets which had not flowered	2141 (26.3%)	2660 (17.3%)

as in 1957. In both years the number of midges emerging in transit was small compared with the total number of midge larvae and pupae which were present in the samples, and in 1958 fewer adults were collected despite the increased number of samples. The average number of larvae and pupae recorded in a

TABLE V.

Number of non-flowered spikelets and the average number of spikelets containing midge in 50 spikelet sub-samples.

Number of spikelets which had not flowered	1957		1958	
	Number of samples	Average number of spikelets con- taining midge	Number of samples	Average number of spikelets con- taining midge
0-5	52	5.13	167	2.17
6-10	26	2.00	52	1.02
11-15	24	0.50	31	1.64
16-20	20	0.95	14	0.50
21-25	15	0.13	15	0.80
26-30	15	0.00	12	0.33
31-35	6	0.83	4	0.00
36-40	4	0.00	6	0.00
41-45	1	0.00	1	0.00
46-50	0	—	5	0.00

spikelet agrees with the observed condition at Samaru where normally one, but occasionally two or three, larvae develop in a single spikelet. The greatest number of larvae and pupae found in a single spikelet during the survey was 13 larvae and one pupa.

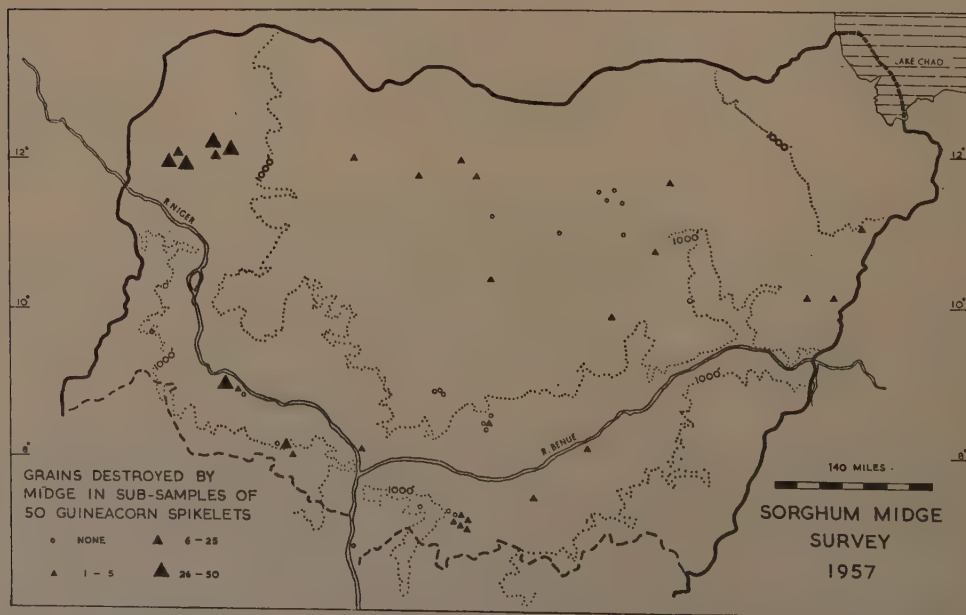


Fig. 3.—Distribution of survey samples and severity of midge attack in 1957.

The figures given in this table for the percentage of spikelets containing midge are not reliable estimates of the grain lost to midge in Northern Nigeria during the two years of the survey but better estimates can be determined by considering all aspects of the survey.

The selection of samples and sub-samples was unbiased, but two other factors, the siting of the sampling areas and the maturity of individual samples, affect the validity of the results. Of these, the maturity of the samples is of greatest importance since from the counts of non-flowered spikelets it is apparent that many samples were taken too early and, since the midge oviposits after flowering, such samples were not fully exposed to midge attack and cannot therefore give an accurate estimate of the level of infestation. The relationship between the



Fig. 4.—Distribution of survey samples and severity of midge attack in 1958.

maturity of the samples and the proportion of spikelets containing midge is shown in Table V.

The correction of these data to a constant number of non-flowered spikelets is not permissible since the basic immaturity of the samples would remain uncorrected and therefore all samples whose sub-samples contained more than five unflowered spikelets were discarded.

The distribution of the remaining samples and the severity of midge attack in each sample are shown in figs. 3 and 4 and the intensity of guineacorn production in Northern Nigeria is indicated in fig. 5, which is based on the distribution of population, the location of forest reserves (in which little or no guineacorn is grown) and estimates of the production of each province in 1958. The estimates obtained for the severity of midge attack in each province are summarised in Table VI.

In 1957, few samples were taken and they were poorly distributed. No over-all assessment of crop losses can be made but the particularly severe midge attack in Sokoto province is worth comment. Agricultural officers in the province had expected a record crop but, at harvest, yields were well below expectation. This

TABLE VI.
Severity of midge attack and order of crop losses in Northern Nigeria in 1957 and 1958.

Province	1957		1958					
	Number of samples	% spikelets containing midge	Number of samples	% spikelets containing midge	*Estimated guineacorn production, 1958 (tons grain)	Estimated loss of grain to midge (tons grain)	* Estimated average yield per acre (lb. grain)	Estimated acres of crop lost to midge
Adamawa ..	2	5.0	6	5.7	264000	15800	1156	30600
Bauchi ..	9	0.4	13	2.0	252000	5100	1060	10700
Benue ..	16	3.2	7	1.2	103000	1200	759	3500
Bornu ..	2	6.0	16	13.0	220000	32800	840	87200
Ilorin ..	5	14.0	15	11.9	53000	7100	826	19200
Kabba ..	6	5.7	9	9.8	68000	7400	979	16900
Kano ..	3	1.3	34	2.2	482000	11000	958	25700
Katsina ..	1	4.0	9	2.9	246000	7300	1027	15900
Niger ..	0	—	22	0.9	135000	1200	742	3600
Plateau ..	0	—	7	1.1	63000	700	664	2300
Sokoto ..	7	51.1	5	2.0	67000	1400	1307	2400
Zaria ..	1	2.0	24	0.1	153000	100	1011	200
	Mean: 10.5		Mean: 4.1		2106000	91100		218200

* Derived from 'Report on the sample census of agriculture 1950-51', Dept. of Statistics, Lagos, and 'Crop production estimates 1956/57 to 1958/59', Dept. of Statistics, Kaduna.
Dry-season guineacorn, which is not attacked by midge, is not included in the estimate of production for Bornu.

was attributed to heavy late storms but reports mention the appearance of sterile heads in the crop, which, together with the survey results, suggests that sorghum midge was partly responsible for the loss of yield but that its activities were not recognised.

In 1958, the survey samples were well distributed through the main guineacorn-producing areas and the results have therefore been used to estimate the magnitude of crop losses (Table VI).

Assuming an average value of £20 per ton, the over-all loss of 91,100 tons of grain represents a financial loss of £1,822,000 in Northern Nigeria in 1958. This is undoubtedly an underestimate of the true loss to midge since the survey method did not take account of spikelets from which the midge had emerged or spikelets containing eggs and first-instar larvae and therefore tended to underestimate the proportion of spikelets attacked by midge.

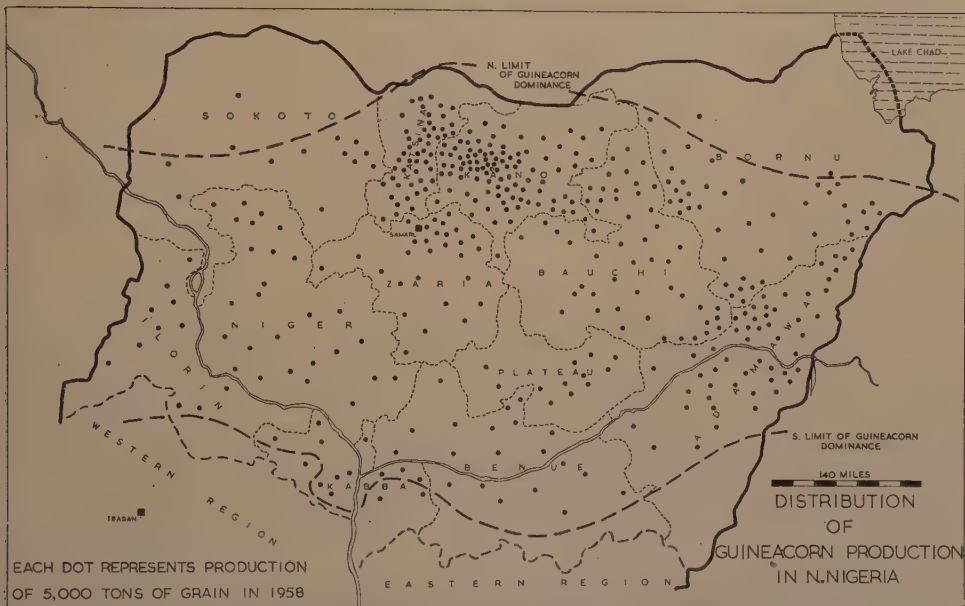


Fig. 5.—Provisional map of guineacorn production in Northern Nigeria.

The unrewarded cultivation of almost a quarter of a million acres is possibly a more realistic expression of the loss due to midge and it is a loss that Northern Nigeria can ill-afford.

The pattern of midge attack in the two surveys suggests that in the areas where most guineacorn is produced the crop suffers a comparatively low attack. Thus, above latitude 9°N . and over the 1,000-ft. contour 1.9 per cent. of all spikelets contained midge in 1957 and 2.8 per cent. in 1958. At lower altitudes and in lower latitudes more severe midge attacks were recorded and these were probably the result of the longer growing seasons, higher humidities and more abundant wild sorghum.

The conclusions drawn from these surveys must be provisional and it is hoped that further surveys may be completed in the near future.

Control of sorghum midge.

The farmers of Northern Nigeria recognise the empty heads which result from severe midge attack but they attribute them to poor fertilisation or to the drying effect of the night breeze which is the forerunner of the dry season. They are quite unaware of the midge itself and are extremely surprised when its presence in empty spikelets is demonstrated.

So long as cause and effect remain unrecognised, any control of midge which depends on the co-operation of the farmer is likely to meet with little success but, with a rapidly rising standard of education, the present barrier to further progress may soon disappear.

Possible control measures are therefore discussed with a view to their future use rather than their present application.

Cultural control.

Given a well-informed farming community, cultural control would be the most natural and effective method of reducing losses to midge. The basic requirements are the destruction of infested heads after harvest, the disposal of threshing trash by burning, burying or composting; the elimination of early-flowering volunteer guineacorn and the growing of a uniform crop with as little spread of flowering as possible. In addition, in some areas it may be necessary to eliminate the wild sorghums which serve as alternative food-plants to the midge.

Cultural control would be least effective in areas where both early- and late-maturing varieties are grown but this practice is not common in the main guineacorn-growing areas.

Chemical control.

At Samaru, the severe midge attack which normally develops on the plant-breeder's varietal collection was controlled in 1959 by spraying each variety as it came into flower. The physical and economic difficulties of applying such a treatment to native farms are insuperable, and there seems little promise of obtaining general chemical control at present. The development of a cheap, safe, adequately persistent systemic insecticide which could be applied to the roots or base of the plant might make chemical control more feasible, but even so it is doubtful whether its application would be either popular or desirable.

Resistant varieties.

The assessment of midge damage on varietal trials reveals marked differences in the level of attack on different varieties, but these differences result from varietal differences in the time of flowering, which may or may not coincide with high populations of midge, rather than from any intrinsic resistance in the varieties.

The only proved resistance to midge known at present is that of the Nunaba group of varieties discovered in the Gold Coast by Bowden & Neve (1953). These varieties, which belong to Snowden's *Sorghum membranaceum*, possess long papery glumes which are not parted by the anthers at anthesis and these characters apparently hinder insertion of the ovipositor by the female midge.

A collection of the Nunaba group of varieties from the Gold Coast has been grown at Samaru and, by creating, artificially, a high population of midge in their vicinity, their resistance has been tested.

In 1959, on four replicates of 15 varieties of the Nunaba group, 0.1-2 per cent. of the spikelets were attacked by midge, and the mean of 0.2 per cent. for these varieties was significantly lower than the over-all mean of 11.5 per cent. for the local non-resistant Farafara. Farafara and the resistant varieties came into

flower together, and it is concluded that the results of the experiment are a true reflection of the relative resistance of Nunaba varieties in the field.

But tests made in the laboratory suggest that the resistance of the Nunaba varieties may break down when the midge is not free to choose between resistant and non-resistant varieties. In each of three experiments, nine females of *C. sorghicola* were confined in a petri dish with five sprigs of spikelets of both Nunaba and Farafara and in a fourth experiment 13 females were confined on ten sprigs of Nunaba. The midges oviposited for more than five hours and when oviposition was complete the number of eggs in random samples of nine spikelets was counted and the width of the ovary was taken as an indication of the stage of development of each set of spikelets. The results are given in Table VII.

TABLE VII.

Laboratory observations on the oviposition of *C. sorghicola* on resistant and non-resistant guineacorn.

		No. of spikelets exposed to midge	Total eggs laid in samples of 9 spikelets	Width of ovary in samples (mm.)
Experiment 1				
Farafara	..	35	70	2.35 ± 0.25
Nunaba	..	59	1	2.28 ± 0.26
Experiment 2				
Farafara	..	46	17	3.38 ± 0.40
Nunaba	..	76	1	3.31 ± 0.27
Experiment 3				
Farafara	..	30	22	2.72 ± 0.26
Nunaba	..	39	27	1.09 ± 0.05
Experiment 4				
Farafara	..	0	—	
Nunaba	..	105	14	2.54 ± 0.55

In Farafara, anthesis occurs when the ovary is between 1 mm. and 2 mm. wide and the midge oviposits most readily on spikelets with an ovary width of just over 2 mm. Thus, in experiment 1, the Farafara spikelets were in the most suitable condition for oviposition and significantly more eggs were laid on the non-resistant variety. In experiment 2, the Farafara spikelets were less suitable but the development of the Nunaba spikelets was also advanced and the midge again laid significantly more eggs on the non-resistant variety. In experiment 3, the Farafara spikelets were again past the optimum for oviposition but the Nunaba spikelets were considerably younger and the midges oviposited equally on both varieties and finally, with no choice of varieties the midge oviposited on Nunaba in experiment 4.

Both in these experiments and in other observations it has been noted that the female midge persists in its attempts to oviposit even under the most unfavourable conditions, and the fact that the ovipositing habit of the female can be adapted to changing conditions should be taken into consideration in any attempt to breed resistant varieties.

Conclusions.

It is hoped that this study of *C. sorghicola* may serve as a basis for further studies of the pest in Nigeria and elsewhere in West Africa.

Future development of the work should involve further surveys of severity of midge attack, since the assessment of crop losses in a single year cannot be taken as an adequate measure of the recurrent losses. Further studies of *C. sorghicola* on *Andropogon gayanus*, additional experiments on the nature of varietal resistance, and a general extension of the biological observations, which at present are mainly based on Samaru, are also desirable.

Summary.

Sorghum midge, *Contarinia sorghicola* (Coq.), was discovered in Nigeria in 1953, and a survey which indicated the widespread occurrence of the midge in the country was followed by the investigations reported in this paper.

Food-plants of the midge in Nigeria are guineacorn (*Sorghum vulgare*, *sensu lato*), which annually provides about two million tons of grain for human consumption, and the wild grasses, *Andropogon gayanus* and *Sorghum arundinaceum*.

At Samaru, Zaria, Northern Nigeria, midges emerge from infested guineacorn heads in the early morning with maximum emergence between 7.45 and 8.15 a.m. After mating, females fly to recently flowered heads where they lay eggs within the spikelets, laying twice as many eggs on the glumes as on the pales. Each female may lay about 50 eggs and both males and females usually die within ten hours of emergence. The egg hatches within four days and, after ten days' feeding, during which the ovary shrivels up, the larvae pupate within the spikelet. The cycle from egg to adult is completed in 19 to 22 days during the growing season but towards the end of the rains larvae spin cocoons and enter diapause.

Large numbers of diapause larvae are carried through the dry season in late-flowering heads which, because they are severely attacked by midge, are not harvested and remain on the stems which are kept in stacks and used for building and fencing or as fuel. Smaller numbers of larvae are present in threshing trash. The emergence of adults from the diapause population was observed from 1955 to 1959. In cages, the first adults were observed to emerge half way through the rains, about 5 to 7 weeks after the weekly mean R.H. had exceeded 60 per cent. and emergence continued for 9 to 12 weeks. Field observations confirmed experimental observations and showed that the build-up of the midge population before the main crop comes into flower is largely dependent on the presence of early-flowering varieties. So long as sufficient guineacorn is in flower and the weather is favourable the midge population builds up steadily to a peak in October and early November.

A. gayanus appears to be of little importance as an alternative food-plant, and preliminary evidence of the existence of a distinct biological race of *C. sorghicola* on *A. gayanus* is presented. Wild sorghum (*S. arundinaceum*) may be an important alternative food-plant in the south, where it is most abundant, but is of little importance in the main guineacorn-growing areas of the north where it is uncommon. *Eupelmus popa* Gir., *Eupelmus* sp., *Aprostocetus* sp. and two species of *Tetrastichus* parasitise *C. sorghicola*, and examples of the three genera are generally present at Samaru in the ratio 3 of *Eupelmus*:1 of *Aprostocetus*:1 of *Tetrastichus*. They are only of importance late in the season. Two spiders, a species of *Thomisus* and a species tentatively referred to as *Diaea*, prey on ovipositing midges but are apparently of little importance.

Experiments show a significant negative linear regression of yield on the proportion of spikelets attacked. There is no evidence of compensation and estimation of the proportion of spikelets attacked by midge gives a direct measure of the loss of yield. In 1957 and in 1958, random samples of guineacorn spikelets were taken from farmers' crops throughout Northern Nigeria. The proportion of spikelets containing midge larvae and pupae was estimated by dissecting 50-spikelet sub-samples taken at random from each sample, and the maturity of the

sample was measured in each sub-sample by counting the number of spikelets which had not flowered. Samples should have been taken between 7 and 21 days after 50 per cent. of the crop had flowered, but many of the samples were immature and were discarded. In 52 mature sub-samples obtained in 1957, 10.5 per cent. of all spikelets contained midge and, in 167 obtained in 1958, 4.1 per cent. contained midge. In 1957, the results did not permit estimation of over-all crop losses but in 1958 it was estimated that at least 91,100 tons of grain, valued at £1,822,000 and representing the produce of 218,200 acres, were lost to sorghum midge.

The pattern of intensity of midge attack in the survey samples suggests that in the main guineacorn-growing areas, which lie above 9°N. at an altitude exceeding 1,000 ft., midge damage is less severe than in lower latitudes and at lower altitudes where heavier attacks probably result from the longer growing seasons, higher humidities and more abundant wild sorghum.

Nigerian farmers recognise the empty heads caused by midge but are unaware of the midge itself and, until they have learned to recognise cause and effect, control measures depending on their co-operation may be unsuccessful. Cultural control by disposal of crop residues and the growing of a uniformly flowering crop would be the most natural and effective method in the main guineacorn-growing areas. There is little possibility of achieving chemical control at present and, though the field resistance of the Nunaba group of varieties (*Sorghum membranaceum*) from the Gold Coast has been confirmed in Nigeria, laboratory experiments suggest that when the midge is not free to choose between resistant and non-resistant varieties it is able to adapt its behaviour and will then oviposit on resistant varieties.

This paper is intended to serve as a basis for further studies of sorghum midge in Nigeria and elsewhere in West Africa.

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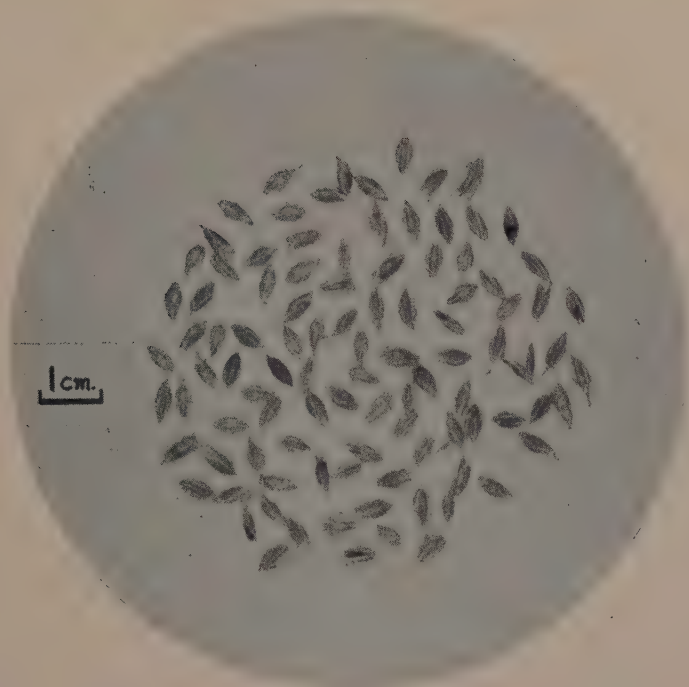


FIG. 1. A hundred guineacorn spikelets that have been attacked by *Contarinia sorghicola*.

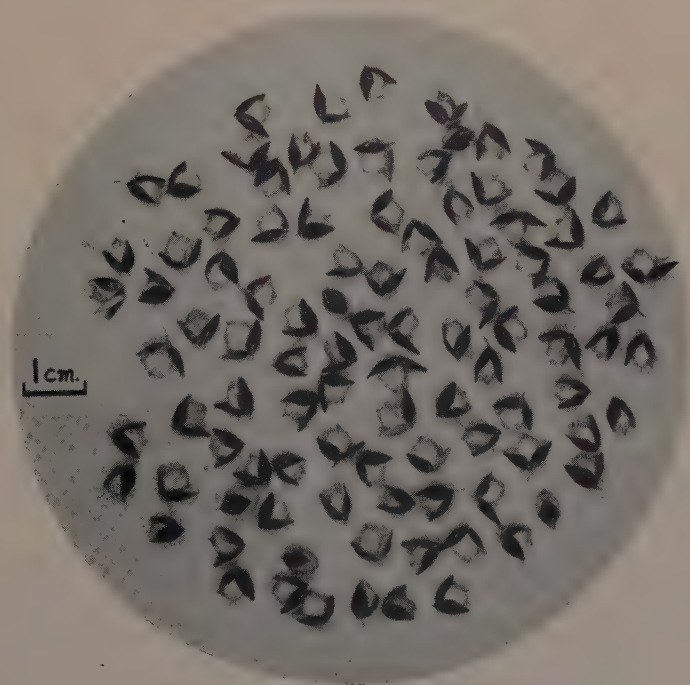


FIG. 2. A hundred healthy spikelets from the same crop.

THE DEVELOPMENT OF *MONOCTONUS PALUDUM* MARSHALL*
(HYM., BRACONIDAE) IN *NASONOVIA RIBIS-NIGRI* ON LETTUCE,
AND IMMUNITY REACTIONS IN OTHER LETTUCE APHIDS.

By D. C. GRIFFITHS

Department of Zoology, University of Durham, King's College,
Newcastle upon Tyne.

E.M.V.

Two main theories have, in the past, been put forward concerning the immunity that certain insects exhibit towards various insect parasites.

The first is the theory of passive resistance which postulates that the parasites fail to develop in certain hosts because these hosts are inadequate as a nutritional medium. The second theory explains suppression of the parasites' development in terms of an active resistance on the part of the hosts which can take the form of either (a) a humoral effect whereby the parasites succumb to chemical secretions in the hosts' body fluids, or (b) a cellular effect which may be either phagocytosis or encapsulation, the latter sometimes accompanied by a process of melanisation.

Bess (1939) and Muldrew (1953) review the evidence in the literature supporting these theories, and since the publication of these papers the most important contributions to this problem have been those of Salt (1955, 1956, 1957), which have demonstrated the widespread occurrence of encapsulation and melanisation as a means of defence against eggs and larvae of the Ichneumonid, *Nemeritis*, artificially injected into species of Lepidoptera, Diptera, Coleoptera and Orthoptera.

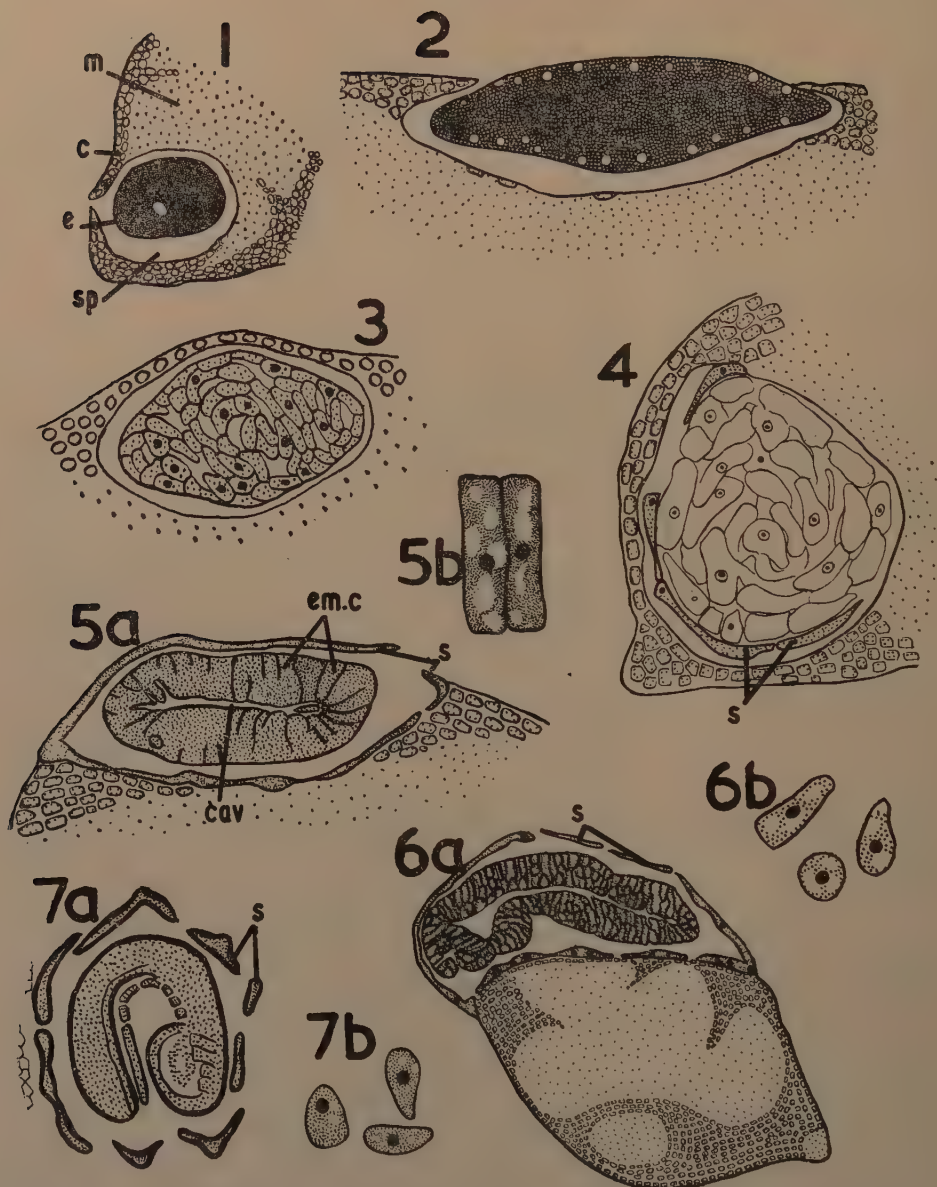
No previous work has been done, however, on the defence reactions of aphids to insect parasites, the most closely related study being that of Bess (1939) on the resistance of mealybugs to parasitisation. He showed that only in some resistant hosts does the parasite become enveloped in a dense phagocytic cyst while in other resistant hosts such eggs and larvae are seldom phagocytosed. His view, therefore, was that immunity is not necessarily accompanied by phagocytosis in these insects although he did not commit himself as to the nature of the immunity reaction in those cases where phagocytosis did not occur.

It was the view of Flanders (1934) that cellular or phagocytic immunity does not occur in aphids because the number of blood cells is relatively few, and Salt (1956) states that blood cells in exopterygote nymphs are fewer than in the adults. It is of interest, therefore, to determine what type of immunity reaction is in fact exhibited by aphid nymphs which are attacked by insect parasites.

The association between the parasite *Monoctonus paludum* Marshall and various species of aphids which occur on lettuce provides a suitable opportunity for the study of such immunity reactions. This parasite is known to oviposit indiscriminately into nymphs of *Nasonovia ribis-nigri* (Mosley), *Aulacorthum solani* (Kalt.), *Macrosiphum euphorbiae* (Thos.), *Myzus persicae* (Sulz.) and *Aulacorthum circumflexum* (Buckt.) on lettuce (Griffiths, 1960) although its eggs only complete their development in *N. ribis-nigri*. The opportunity is presented, therefore, of studying the normal development of the parasite in its true aphid host, and

* Starý (1959) in a revision of the genus *Monoctonus* identifies *M. paludum* Marshall with *M. crepidis* (Hal.) which has been recorded from other aphid hosts on plants related to lettuce.

As the present paper deals only with the relationship between the parasite and lettuce aphids the terms "normal host" and "true host" refer specifically to *Nasonovia ribis-nigri* (Mosley) on lettuce.



Figs. 1-7.—Development of the parasite in its true host, *N. ribis-nigri*. 1. Immediately after attack. c, nerve tissue cortex; e, parasite egg; m, nerve tissue medulla; sp, space separating parasite from nerve tissue of host (x 425). 2. Six hours after attack. The nuclei can be seen around periphery of the egg (x 425). 3. Eleven hours after attack. The cytoplasm has by now divided to produce a segmented embryo (x 425). 4. Seventeen hours after attack. The serosa (s) can be seen separating off as a flattened layer of cells to the outside of the embryo (x 660). 5a. Twenty-four hours after attack. The serosa (s) surrounds an embryo which consists of columnar cells (em.c) arranged about a central cavity (cav) (x 425). 5b. Columnar embryo cells (approx. x 1600). 6a. Thirty-six hours after attack. The parasite embryo has now come to lie adjacent to the nervous tissue, suspended in the sac-like serosa (s). The central cavity is now surrounded by a layer of cells 2 to 3 cells deep (x 320). 6b. Embryo cells at 36 hours (approx. x 1600). 7a. Two days after attack. Parasite embryo lying in body cavity of host, surrounded by serosal envelope (s) (x 250). 7b. Embryo cells at two days (approx. x 1600).

comparing this, stage by stage, with the processes which suppress its development in the other four aphid species.

Material and methods.

Lettuce plants were grown in an allotment in the Newcastle-upon-Tyne district during 1958-59, and parasitised material of *N. ribis-nigri* was collected from the plants in order to obtain a supply of emerging parasites.

It is known that unfertilised females of *M. paludum* give rise to male progeny only whereas fertilised females produce both males and females. For the purpose of these experiments, therefore, only unfertilised females were used, so that the descriptions refer always to the development of male parasites.

The lettuce aphids which were used in the experiments had been bred in laboratory cages, free from parasite infestation. No artificial injection technique was needed and all that was necessary was to confine freshly emerged female parasites with half-grown aphid nymphs, whereupon attack would readily commence. Aphids which had been attacked were removed to separate dishes, each containing a piece of leaf, and placed in a constant-temperature room at 26°C.

Sample groups of these attacked aphids were then fixed in warm (70°C.) Bouin's solution at various time-intervals after attack. Larval stages were dissected out of the aphids and stained in borax carmine but, for the younger stages of parasitic development, the aphid hosts were embedded, using Peterfi's celloidin-paraffin technique (Pantin, 1946) and then sectioned at thicknesses of 2 μ to 10 μ , the sections being stained in either iron haematoxylin, trioxyaematin or Mayer's haemalum and eosin.

Results.

The sections revealed a general similarity of internal structure in all the aphids studied and in all of them the form of the nerve ganglia is that which is shown in fig. 32. It has been shown (Griffiths, 1960) that *M. paludum* lays a single egg in each aphid by inserting the ovipositor with precision into the ventral suture between the aphid's first and second pairs of legs. Because of this, the egg automatically comes to lie in the posterior thoracic mass of fused ventral nerve ganglia of the host. Cases of other parasite eggs being deposited inside the nervous tissue of the host have been recorded by Marchal (1906) and by Strickland (1930).

Normal development of M. paludum in the true host species, N. ribis-nigri.

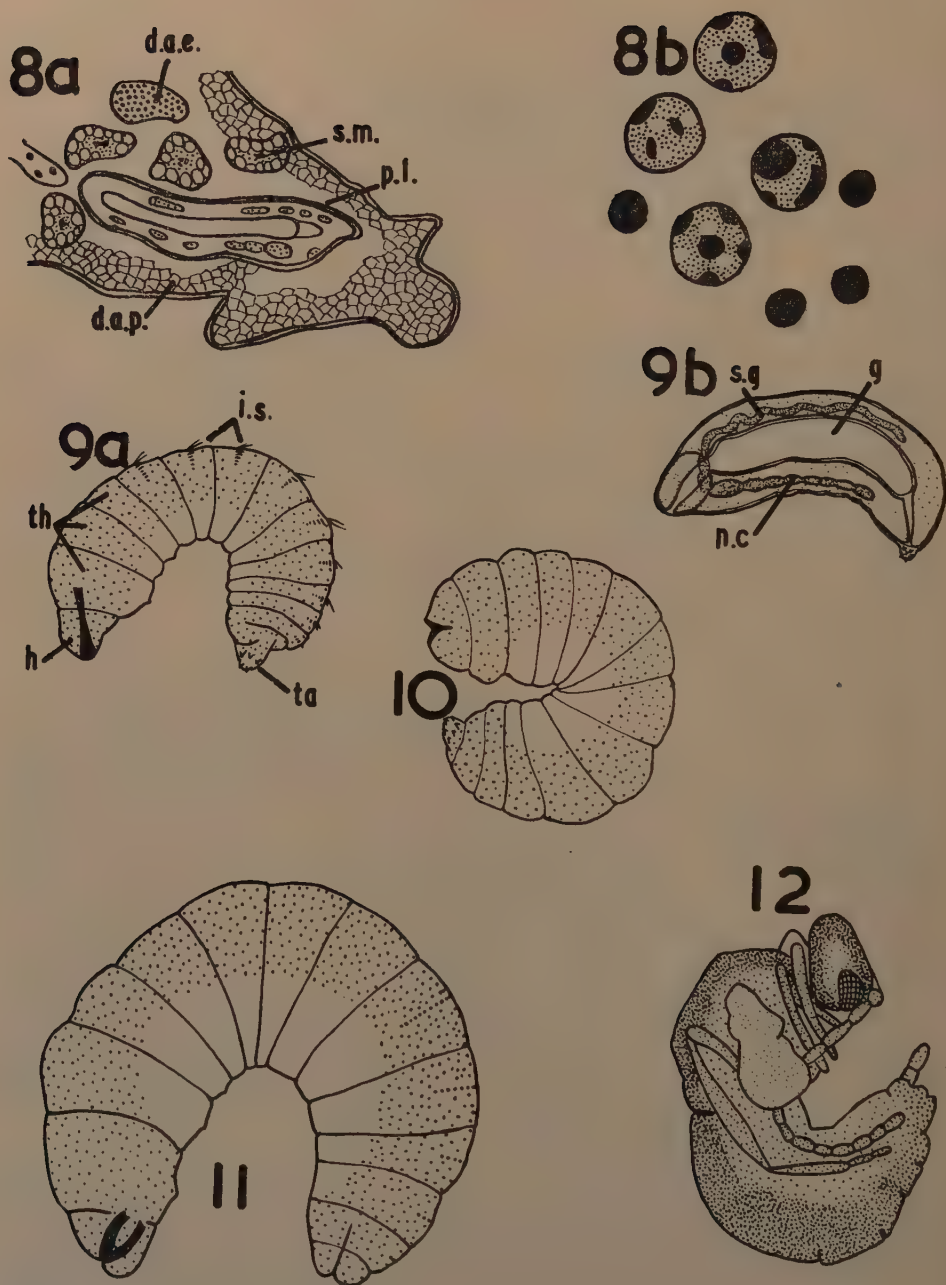
Sections of those aphids which were fixed immediately after attack by the parasite (fig. 1) revealed an egg embedded in the nerve ganglia of each host. The eggs were conspicuous because they stained very deeply with the haemalum stain and each was surrounded by a clear space which separated it from the surrounding nervous tissue. The eggs presented a finely granular appearance, and it was not normally possible to see the close-fitting chorion.

Sections of aphids which were fixed at six hours after attack revealed that changes had taken place in the eggs. By this time the nucleus of each egg had divided and the resulting daughter nuclei had come to take up a position around the periphery of the egg (fig. 2).

By 11 hours the nuclei had divided further and the cytoplasm had divided also, so that the embryo then consisted of a mass of rather irregularly arranged cells (fig. 3).

By the time 17 hours had elapsed, a flattened layer of cells had begun to be cut off on the outside of each embryo (fig. 4). (This layer of cells later became the trophamnion or serosa.)

At 24 hours (fig. 5a) each embryo had the appearance of a regularly arranged row of more or less columnar cells (fig. 5b) surrounding a central cavity. The



Figs. 8-12.—Development of the parasite in its true host, *N. ribis-nigri* (cont.). 8a. Two-three days after attack. First-instar larva which has now freed itself of its serosa. d.a.e., degenerating aphid embryo; d.a.p., degenerating aphid adipose tissue; p.l., parasite larva; s.m., serosal mass (x 85). 8b. Degenerating aphid embryo cells (approx. x 2600). The larger cells possess vacuolated cytoplasm and disorganised nuclear material, and, in the smaller ones, the cytoplasm has been withdrawn so that only nuclear material remains. 9a. Four days after attack. Second-instar larva. h, head; i.s., integumentary spines; ta, short, spinous tail; th, thorax (x 70). 9b. Four days after attack. Second-instar larva stained in borax carmine. g, gut; n.c., nerve cord; s.g., salivary gland (x 40). 10. Four-five days after attack. Third-instar larva (x 40). 11. Five-six days after attack. Last-instar larva (x 40). 12. Seven days after attack. Pupa and meconium of *M. paludum* (x 40).

serosa was now well developed and formed a syncytial layer of deeply staining cells separating the embryo from the surrounding nervous tissue. It is of interest to note that, although various changes had been going on in the embryo, there was no appreciable increase in size up to this stage.

It was in the stages succeeding the 24th hour, *i.e.*, after the formation of the serosa, that increases in size were most apparent. By the 36th hour, the embryo had lengthened considerably and some degree of folding had taken place (fig. 6a). The cells surrounding the central cavity had undergone division so that they were now two or three layers in depth (fig. 6b) and smaller than those of the previous stage. The future anterior end could now be recognised by its larger size, and it could be seen that the embryo had, by this time, come to lie outside the nervous tissue, suspended in the sac-like serosa. The serosa itself was fully differentiated and consisted of an envelope of flattened cells with prominent nuclei and a clear cytoplasm which had taken up the haematoxylin stain, and which could be seen to contain a few small vacuoles (fig. 29a).

In these and in later sections, blood cells of the host could sometimes be seen attached to the serosa (fig. 31a-c), just as they were attached to other tissues of the aphid's body. These cells were of two main types: firstly, small rounded cells, less than 10μ in diameter, with a granular, vacuolated cytoplasm, and a nucleus which was in some cases rather pale, and secondly, larger spindle-shaped or pyriform cells, $20-30\mu$ in diameter, with a clear non-vacuolated cytoplasm, and a large prominent nucleus containing a large nucleolus. (The small cells may correspond to the phagocytes described by Hollande (1911) in the aphid, *Lachnus*.) Although these cells came into close contact with, and in some cases appeared to penetrate the serosa they never collected around the serosa in great numbers, and presumably they did not seriously interfere with its normal functioning.

Growth and division continued at a rapid rate, producing a 48-hour embryo (fig. 7a), approximately 0.33 mm. long. The embryo cells, a few of which are shown in fig. 7b, were as yet undifferentiated, but they were organised into distinct masses, which occupied positions corresponding to the future organ systems of the body. The embryo lay well outside the nervous system, and, in the serosa which surrounded it, changes had begun to take place. The nuclei of the serosa had expanded into more elongated masses of chromatin material, and the vacuoles in the cytoplasm were larger and more conspicuous (fig. 29b). The nuclear material then began to dissociate into separate pieces which could be seen as small, darkly staining bodies, each surrounded by an area of clear protoplasm (fig. 29c). Between the second and third day, the embryo further increased in length and broke free of its serosa, and, in longitudinal section (fig. 8a) of this first-instar larva, it could be seen that the larval organs were well differentiated. Around the larva were several serosal masses (fig. 8a (s.m.) and fig. 29d) each of which had been formed by the confluence of nuclear material in the centre and the cytoplasm around it, the peripheral region being occupied by the greatly enlarged vacuoles. These observations on the development of the serosal masses are similar to those of Spencer (1926) and further confirm the views of Jackson (1935) concerning the origin of these peculiar structures in parasitised insects.

Degenerative changes were apparent at this stage in the adipose tissue of the host and in the aphid embryos. It appeared that the cytoplasmic material was withdrawn from them, resulting in the production of a vacuolated and irregular mass in the former case, and of a shrinkage and loss of shape in the latter (fig. 8a (d.a.e.)), so that the cells of the aphid embryos came to contain a mass of rather disorganised nuclear material together with a very small amount of vacuolated cytoplasm. Cells of this type are shown in fig. 8b. Spencer considers that these changes are brought about by a cytolytic or digestive enzyme secreted by the parasite and that the products of digestion are then absorbed by the developing parasite.

Further rapid growth produced, by the fourth day, larvae which could easily be dissected from their hosts, and which were almost 1 mm. in length. A representative second-stage larva is shown in figs. 9a and 9b. There was a distinct head, which projected dorsally above the mouth, and behind this were three thoracic segments and ten abdominal segments. The dorsal surface of the larva was covered with bristles which were particularly well developed as a dorsal band in the mid-region of each abdominal segment—the so-called integumentary spines (Beirne, 1942). The tail was short and blunt and covered with small spines. The gut and associated structures were by now well developed and the presence of a sclerotised jaw apparatus and of pieces of partially-digested material in the gut showed that the larva had now begun to feed by the mouth.

The third-stage larva, which developed between the fourth and fifth day, was again much larger than the previous stage (fig. 10). It lay in a curled position, occupying the greater part of the space in the aphid's body, and, in appearance, was similar to the previous larval stage, except that the integumentary spines were not so conspicuous. By feeding upon the remaining contents of the aphids' body cavities, third-stage larvae increased rapidly in size before moulting. All the resulting larvae, at the fifth to sixth day, were greatly swollen and completely occupied the space inside the empty aphid shells. A representative fourth-stage larva is shown in fig. 11: the head of the larva recedes dorsally above the mouth-parts; spines are lacking from the tail, and there are no integumentary spines. This is the last larval stage and, by spinning its cocoon on the sixth day, it converts the aphid into the characteristic 'sessile' form, with a shiny, brown and swollen appearance.

Inside the sessile aphids, the larvae pupated (fig. 12), and adult parasites emerged on the eleventh day at the temperature at which these experiments were conducted, *i.e.*, at 26°C.

Abortive development of M. paludum in other lettuce aphids.

Although there was a certain amount of individual variation in the speed at which the developmental processes of the parasite proceeded in the true host species, *N. ribis-nigri*, there was, on the whole, a uniformity of pattern which enabled one to recognise easily the events which were taking place and the approximate duration of each process. In the other species of aphids, however, the individual variation appeared to be much greater, and for this reason, interpretation of the results was more difficult.

At least five individuals of each species of aphid were examined at each stage, any differences between them being noted in the text.

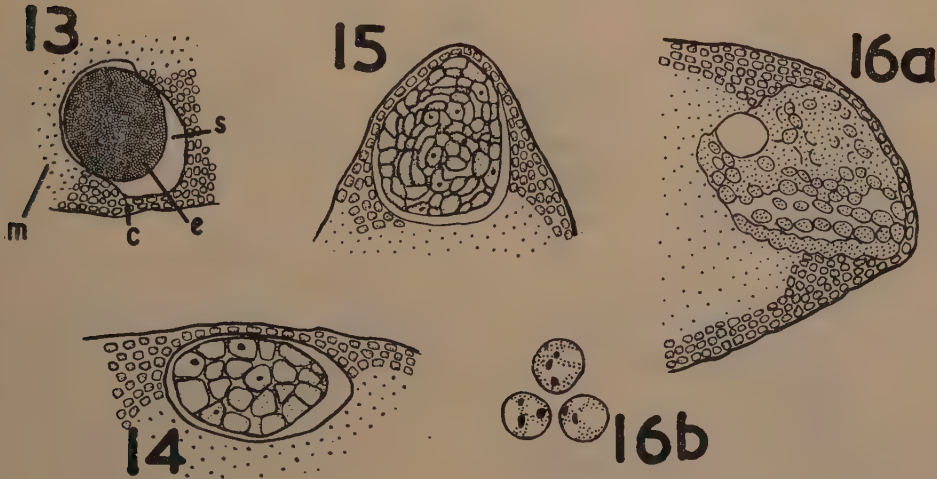
As the behaviour of *M. paludum* is similar towards all species of lettuce aphids used in these experiments, and as the internal anatomy of the aphids is also similar, the egg was invariably deposited in the posterior thoracic nerve mass, and commenced its development there.

(a) *Abortive development of the parasite in Aulacorthum solani.*—An egg of *M. paludum* which has been deposited in the nervous tissue of *A. solani* is shown in fig. 13. In all the individuals examined, the eggs were perfectly normal, and later sections revealed that they developed in the same manner as those laid in *N. ribis-nigri* by division of the nucleus, followed by division of the cytoplasm. The segmented embryos that were produced by the 11th hour (fig. 14) were not, however, so advanced as those in the true host species at a corresponding stage. Development continued at a slower rate than in *N. ribis-nigri*; after 17 hours had elapsed, no serosas had been formed, and at the end of 24 hours the embryos had only reached a stage of development (fig. 15) comparable with that attained in *N. ribis-nigri* by the 11th hour.

Development of the embryos in *A. solani* rarely proceeded beyond this partly segmented condition, for, during the second and third days, the embryos began to

lose their regular shape (fig. 16a), and degenerative processes ensued: the internal contents of the embryos became vacuolated and spaces developed between the cells, the latter becoming shrunken and appearing to consist of vacuolated cytoplasm and disorganised nuclear material (fig. 16b). Accompanying the general shrinkage of the parasites, the nervous tissues of the hosts began to encroach upon the spaces formerly occupied by the embryos. This resulted in the gradual disappearance of the embryos, and the return of the nervous tissue to a normal condition so that, by the fourth day, little, if any, trace of the parasites' attacks could be detected.

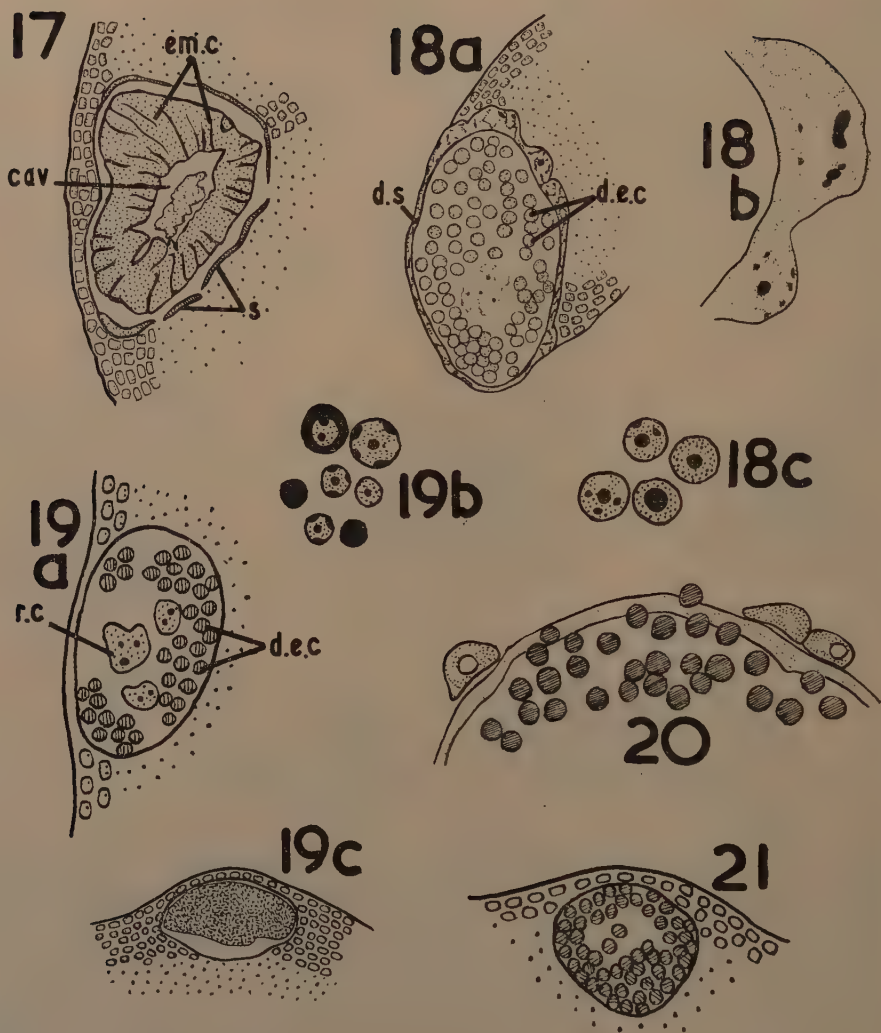
(b) *Abortive development of the parasite in Macrosiphum euphorbiae*.—Each parasite egg was again laid in the nervous tissue of its host, and during the early stages there was little evidence of the general slowness and lack of vigour in



Figs. 13-16.—Abortive development of the parasite in *Aulacorthum solani*. 13. Immediately after attack. c, nerve tissue cortex; e, parasite egg; m, nerve tissue medulla; s, space separating parasite from nerve tissue of host (x 425). 14. Eleven hours after attack (x 425). 15. Twenty-four hours after attack (x 425). 16a. Two-three days after attack. Degenerating parasite embryo still lodged within nervous tissue of its host (x 425). 16b. Parasite embryo cells in *A. solani* at two-three days (approx. x 1600), showing vacuolisation of the cytoplasm and disorganisation of the nuclear material.

development displayed by the parasites in *A. solani*. Segmentation of the eggs took place just as rapidly as in the true host, *N. ribis-nigri*, and serosa formation followed the same course, so that perfectly normal embryos were found 24 hours after attack (fig. 17).

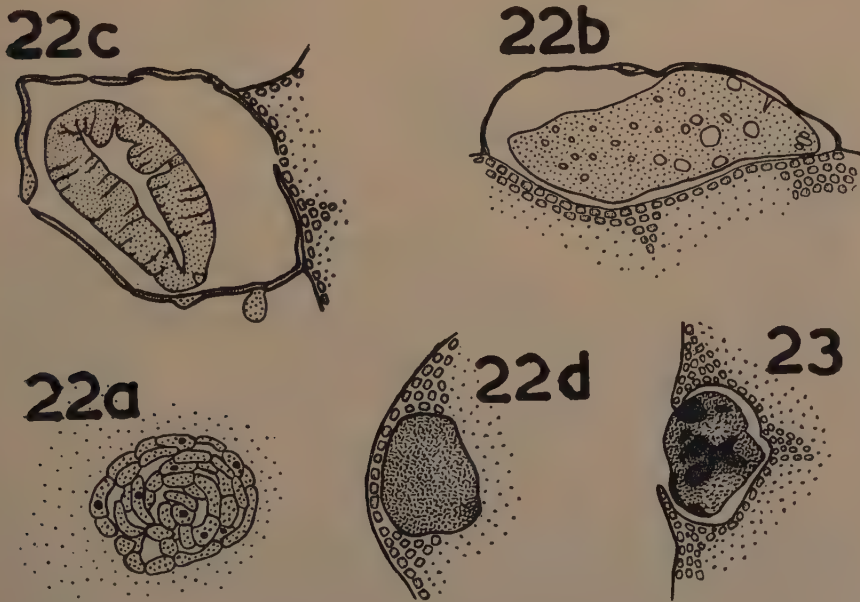
But by the 36th hour, growth of the embryos had been checked and degenerative processes had set in (fig. 18a). There had been no increase in size over the 24-hour condition, and the embryos had not come to lie outside the nervous tissues, as they do in the true host. In none of the individuals examined had the serosa grown at all, and it presented a quite different appearance from the serosa in the true host at an equivalent stage. Instead of being a conspicuous, densely staining structure with prominent nuclei and only small vacuoles in the cytoplasm (cf. fig. 29a), the serosa in *M. euphorbiae* (fig. 18b) was greatly vacuolated at an early stage, the cytoplasmic contents being much reduced, and the whole structure appearing far less conspicuous, with the exception of the nuclear material, which presented a rather disorganised appearance.



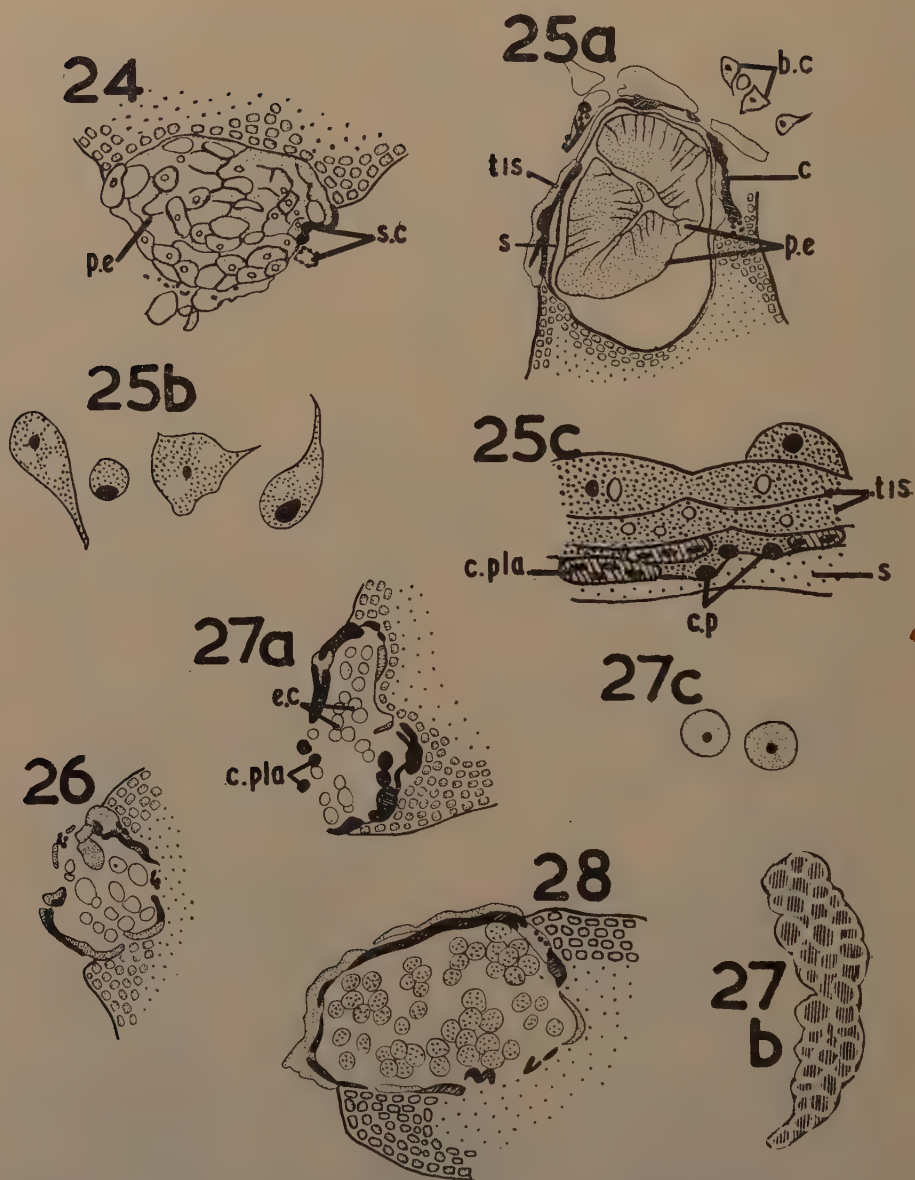
Figs. 17-21.—Abortive development of the parasite in *Macrosiphum euphorbiae*. 17. Twenty-four hours after attack. cav, central cavity; em.c, columnar embryo cells; s, serosa (x 425). 18a. Thirty-six hours after attack. Degenerating parasite embryo still lodged in nervous tissue of host. d.e.c, degenerating embryo cells of parasite; d.s, degenerating serosa (x 425). 18b. Degenerating serosa of parasite (x 1500) showing extreme vacuolisation of cytoplasm. 18c. Degenerating parasite embryo cells (approx. x 1600). These cells are small, i.e., approx. 4μ in diam., and they contain very little cytoplasm and a mass of disorganised nuclear material. 19a. Two days after attack. d.e.c, degenerating embryo cells; r.c, residual cytoplasm (x 425). 19b. Degenerating embryo cells (approx. x 1600). Many of these cells are smaller than those at thirty-six hours and the withdrawal of the cytoplasm results in their containing practically nothing but densely staining nuclear material. 19c. Two days after attack. Degenerate embryo with speckled, granular appearance (x 425). 20. Three days after attack. The serosa in this specimen has persisted, and, associated with it, are small blood cells of the host (x 800). 21. Four days after attack. The degenerating embryo is formed of a closely-packed ball of tiny, darkly staining cells, which contain only nuclear material (x 425).

Concomitant with these changes in the serosa, changes had occurred in the embryo cells themselves. These had become more rounded and more deeply staining (fig. 18c), and their regular arrangement around a central cavity had become obscured. By the second day, further degenerative changes had taken place, the embryonic cells having shrunk still more (figs. 19a-b). Many of these cells now measured little more than 2μ in diameter, and contained practically no cytoplasm, the nuclear material staining very deeply and giving the cells the appearance of dark spots. The serosa had completely disappeared in many of these sections, although it persisted as a flattened vestige in some. One of the ten sections cut at this stage (fig. 19c) presented a rather different appearance from the others, the degenerate embryo being much smaller, and having a dark, granular and rather 'speckly' appearance owing to scattered nuclear material being spread throughout the embryo. This appearance is similar to that of many of the degenerating parasite embryos in the species *Myzus persicae* (described below), and it is thought to be characteristic of those embryos in which degeneration sets in at an early stage of parasite development.

The appearance of sections cut three days after attack differed little from those described at two days, except that several of the aphids contained a large number of blood cells, some of which had come into contact with the flattened serosae of the parasite embryos (fig. 20). It is thought that the blood cells are unlikely to have caused the changes which had occurred in the serosae because (a) these changes were initiated well before this stage, (b) blood cells were sometimes found in association with the serosa in the true host, *N. ribis-nigri* (cf. figs. 31a-c), where they apparently did no harm, and (c) there was no particular concentration



Figs. 22-23.—Abortive development of the parasite in *Myzus persicae*. 22. Twenty-four hours after attack (all $\times 425$). 22a. First type of parasite embryo, showing retarded condition of segmentation. 22b. Second type of embryo, showing pale, vacuolated structure with no sign of segmentation. 22c. Third type of embryo, corresponding with equivalent stage in true host. 22d. Fourth type of embryo, showing scattered nuclear material which gives it a 'speckly' appearance. 23. Two days after attack. Degenerating parasite embryo still lodged in nervous tissue of its host. The scattered nuclear material has condensed in places to form very darkly staining masses ($\times 425$).



Figs. 24-28.—Abortive development of the parasite in *Aulacorthum circumflexum*. 24. Seventeen and a half hours after attack. p.e, segmenting parasite embryo; s.c, scattered capsule tissue (x 425). 25. Twenty-four hours after attack. 25a. Development of capsule around parasite embryo. b.c, blood cells of host; c, capsule; p.e, parasite embryo; s, serosa of parasite; tis, tissue formed by coalition of blood cells of host (x 425). 25b. Blood cells of host (approx. x 1600). 25c. Formation of capsule (approx. x 2000). c.p.la, plates of capsule material; c.p, small pieces of capsule material (other labelling as above). 26. Thirty-six hours after attack. The capsule has, by now, spread around so as to enclose, almost completely, the embryo, which has not increased at all in size over the 24-hour condition (x 425). 27. Two days after attack. 27a. The capsule now extends right around the degenerating parasite embryo, and small pieces of the capsule which have broken away during sectioning (c.p.la) are of about the same diameter as the cells which formed them; e.c, degenerating embryo cells (x 425). 27b. A large piece of capsule material which has broken away and which shows the essential cellularity of its structure (x 1280). 27c. Parasite embryo cells (approx. x 1500). 28. Four days after attack. Degenerating parasite embryo. There has been no change over the two-day condition and the embryo cells have not become shrunken (x 425).

of blood cells around the parasite embryos, these cells being numerous throughout the whole of the aphid bodies. The sections which were cut at 2μ were a little misleading, as they showed the embryonic cells in a scattered array, whereas thicker sections, cut at an identical stage, showed that degenerate embryo cells were arranged in a tight ball. As this ball of cells shrank, the nervous tissue of the aphid encroached upon the space which originally separated the developing parasite from it, so that the typical appearance at four days after attack is that shown in fig. 21.

From six days onwards, it was exceptional to find any trace of a parasite remaining in the aphid tissues, and it was assumed that by this time the parasite embryo had been completely absorbed by the host. There was one notable exception to this; in one individual of *M. euphorbiae*, sectioned six days after attack, a large larva was found, corresponding in size to larvae found in the true host at about two or three days after attack. No explanation could be found for the exceptional development of the parasite in this individual.

(c) *Abortive development of the parasite in Myzus persicae*.—A single parasite egg was laid in the nervous tissue of each host just as in the other species of aphids already described, and segmentation commenced as before. The rate at which segmentation proceeded was, in some cases, slower than that of the corresponding processes in *N. ribis-nigri* so that there was considerable individual variation in the stage which segmentation had reached before the degenerative processes set in. In the ten individuals sectioned 24 hours after attack, the variation was of four different types; the first was exemplified by two embryos which exhibited the retarded condition of segmentation shown in fig. 22a, corresponding to the 11-hour condition in *N. ribis-nigri*; the second type by two embryos which appeared as pale vacuolated structures (fig. 22b) with no sign of segmentation; the third type by two embryos which were normal (fig. 22c), complete with serosa; and the fourth type (fig. 22d) by four embryos which appeared to consist of closely packed fragments of disorganised nuclear material, forming a rather reticulate structure which appeared to contain little cytoplasm.

Owing to the failure of laboratory stocks, the individuals of *M. persicae* which were used for the 36-hour stages had to be brought in from plants in the field. These were used immediately for attack by *Monoctonus paludum*, but it subsequently transpired that most of these aphids were already parasitised by some other parasite at the time of the experiments, and the results pertaining to this stage had to be discarded.

Of the six aphids sectioned two days after attack, one contained an embryo similar to that in *Macrosiphum euphorbiae* at an equivalent stage, showing degenerative changes in the embryo cells and in the serosa surrounding them. Four had a shrunken and crumpled appearance with an internal structure (fig. 23) resembling those of the fourth type of 24-hour embryo (above), but more deeply staining. The sixth had an indistinct and blotchy appearance, somewhat intermediate between the other two types. Further shrinkage was found to occur during the succeeding days, the nervous tissue gradually encroaching upon the space which the parasite formerly occupied so that, by the ninth day, almost all traces of parasitism had disappeared. The simplest explanation of the different appearances of the embryos in this series of sections is that those embryos which perish early are transformed into the fourth type of structure shown in fig. 22d, and that this 'speckly', degenerate embryo becomes darker-staining as its cytoplasmic contents become further withdrawn (fig. 23), with a resulting concentration of the deeply staining nuclear material. This appearance was typical of the majority of individuals of this species, but those embryos which had developed vigorously up to the 24-hour stage presented a different appearance during degeneration, comparable with that described in *M. euphorbiae* (figs. 17–21), and, in addition, a number of intermediate types was found to exist.

(d) *Abortive development of the parasite in Aulacorthum circumflexum.*—The sequence of events in *A. circumflexum* was strikingly different from that in the other species of lettuce aphids studied. A single egg was laid in the posterior mass of nervous tissue of each host as before and commenced vigorous development,

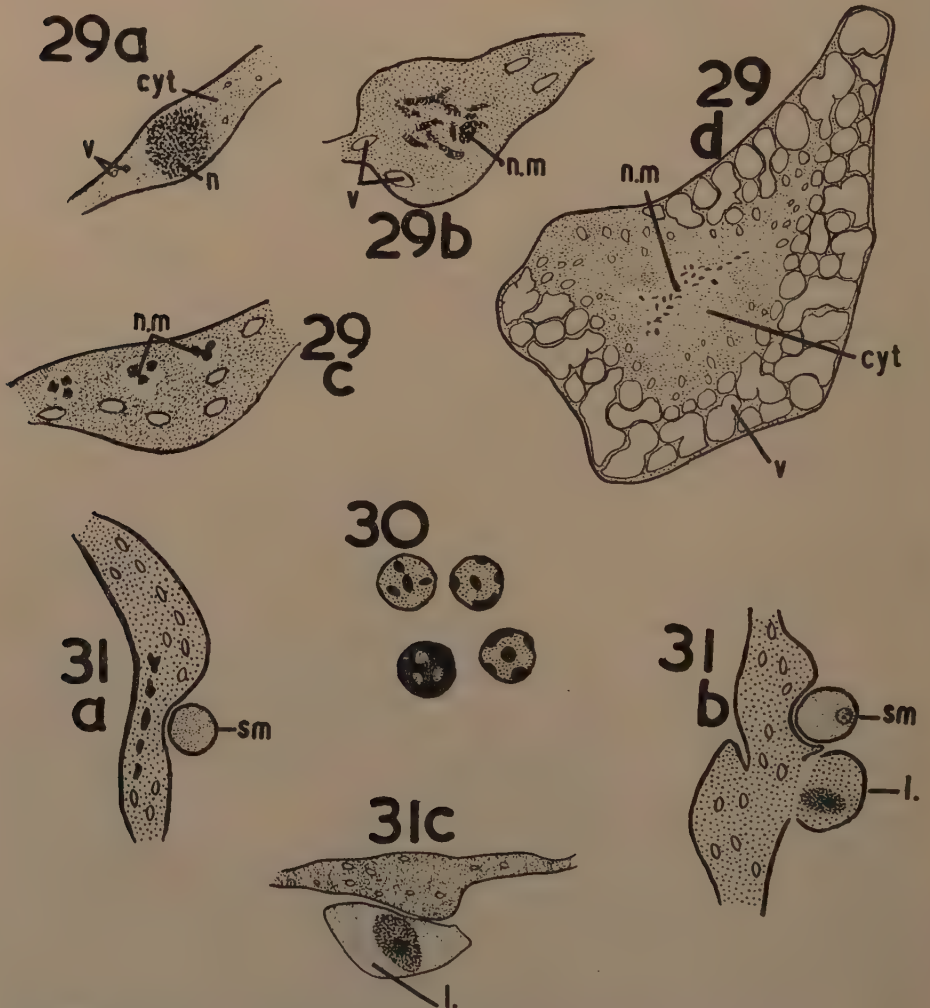


Fig. 29.—Formation of serosal masses in true host, *N. ribis-nigri*. 29a. Normal serosa of *M. paludum* 36 hours after attack, showing large nucleus (n), dense cytoplasm (cyt) and a few small vacuoles (v) (x 1600). 29b. Serosa at 48 hours showing expansion of nucleus into drawn-out masses of nuclear material (n.m.) and increase in size of vacuoles (v) (x 1600). 29c. Serosa at 48 hours showing dissociation of nuclear material (n.m.) into separate masses (x 1600). 29d. Serosal mass at three days after attack. There has been a great increase in size, and the nuclear material (n.m.) is surrounded by the cytoplasm (cyt) which is, in turn, surrounded by the greatly enlarged vacuoles (v) (x 300). Fig. 30.—Partially digested cells from gut of large, unknown parasite larva in *Myzus persicae* (approx. x 1600). The disorganised nuclear material and vacuolated cytoplasm should be noted.

Fig. 31a-c.—Different appearances of the host blood cells in association with the parasite serosa in the true host, *N. ribis-nigri*, 48 hours after attack (all x 700). sm, small blood cell; l, large blood cell.

but this development was arrested by the secretion of a capsule by the host, which effectively isolated the parasite embryo from the host tissues.

The formation of this capsule is well illustrated by sections taken 24 hours after attack. Such a stage is shown in figs. 25a-c, and it can be seen that the parasite embryo has attained a degree of development equal to that attained in the true host at a corresponding stage. Around the embryo in *A. circumflexum*, however, a capsule was observed to form, just outside the serosa of the parasite, beginning at the side of the embryo which faced the body cavity, and later extending to enclose the whole embryo. Its formation appears to be associated with certain of the blood cells of the host, as shown in fig. 25b. These cells are similar in appearance to the small blood cells observed in other species of lettuce aphids, with respect to size and the presence of a vacuolated cytoplasm, but most have a more distinctly pyriform shape, with long pointed processes, and the cytoplasm appears to be less granular.

They appear to be attracted in some way to the parasite embryo, where they then apply themselves to the serosa and spread out to form a thin layer of tissue, with indistinct cell boundaries (fig. 25c (tis)). Each cell then secretes small pieces of capsule material (c.p) which later coalesce to form a plate (c.pla). This plate then joins up with plates formed by neighbouring cells, with the result that by the 36th hour (fig. 26) the embryo is almost completely surrounded by a thin brown membrane, about 4μ in thickness.

The capsule substance is very refringent when looked at in side view, but presents a brown appearance when viewed from above. It tears easily during sectioning and broken pieces of it have a somewhat cellular appearance (fig. 27b). Sometimes the individual elements of the capsule break away (fig. 27a), appearing as brown, roughly circular objects, having the approximate dimensions of the blood cells which formed them.

The earliest signs of formation of the capsule were found in sections taken $17\frac{1}{2}$

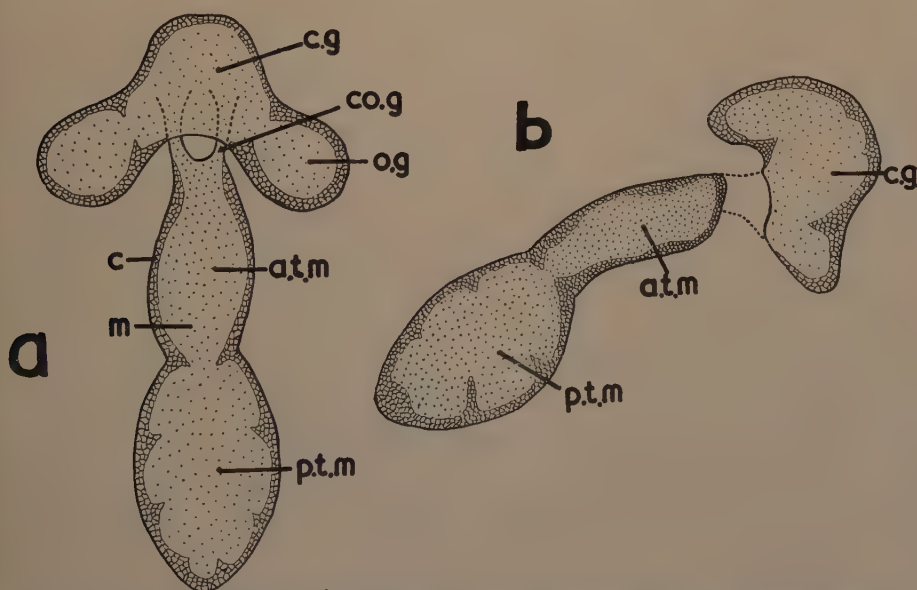


Fig. 32.—Reconstructions of aphid central nervous ganglia (x 85). a, dorsal view; b, lateral view. a.t.m., anterior thoracic mass; c, cortex; c.g., cerebral ganglion; co.g., commissure around gut; m, medulla; o.g., optic ganglion; p.t.m., posterior thoracic mass.

hours after attack. Such an embryo which has developed normally up to this stage, but around which the capsule (s.c) is just beginning to form, is shown in fig. 24. In the early stages of its development, the capsule did not inhibit growth of the embryo, so that the 24-hour stages appeared normal so far as growth was concerned. But, as the capsule became more complete, the growth of the parasite was checked, and, by the 48th hour, the embryo no longer appeared normal, being composed of irregularly arranged, rounded cells (fig. 27c), and occupying no more space than it occupied at 24 hours.

Sections taken four days after attack (fig. 28), showed no alteration of this condition, and even at later stages the embryo cells had not shrunk as much as they had in *M. euphorbiae* but retained a diameter of approximately 5μ , so that, in sections taken eight days after attack, the parasite still appeared as a close-packed ball of rounded cells enclosed by the capsule and lodged in the nervous tissue of its host.

Discussion.

It has been observed that the site for the deposition of the parasite egg is the same in all five species of aphid, and that the egg commences a development which follows the same pattern, no matter which species of aphid is acting as host. But the rate at which such development proceeded, and the stage which it reached before degenerative processes set in, varied between the different species and between the different individuals of each species.

It was common for parasite eggs in *M. euphorbiae* and *A. circumflexum* to develop vigorously up to the 24-hour stage, but less so in *M. persicae* and *A. solani* where, in most cases, the development was arrested at an earlier stage. This variation in the stage at which the degenerative processes set in produced a wide variety of different appearances in the degenerating parasite embryos, ranging from the 'speckly' masses shown in *M. persicae* (fig. 23) to the type of structure consisting of a mass of shrunken cells such as was found in *Macrosiphum euphorbiae* (fig. 19).

The most interesting fact emerging from this study is that at least two different mechanisms were found in these lettuce aphids as a defence against the development of *Monoctonus paludum*. The method employed by *A. circumflexum* appears to be encapsulation of the embryo by products of the blood cells of the host, with consequent isolation of the parasite from supplies of food and oxygen. There was no evidence of the parasite having been killed by any other agent before encapsulation began for, in all cases studied, the parasite development had been vigorous up to that stage. The capsule differs from the relatively thick capsules described by Salt (1955, 1956, 1957) in a variety of other insects as a defence against artificially injected eggs and larvae of the Ichneumonid, *Nemeritis*, but resembles more the thin brown capsules described by Schneider (1950) in the Syrphid, *Syrphus balteatus* (Deg.), as a defence against the Ichneumonid, *Diplazon fissorius* (Grav.).

The mechanism of the degenerative processes in the other false hosts, *A. solani*, *Macrosiphum euphorbiae* and *Myzus persicae*, where no encapsulation process has been observed, is more problematical. The possible explanations to be considered are (i) intracellular phagocytosis of the embryo or of the serosa; (ii) an active, humoral secretion on the part of the host; or (iii) the inadequacy of the host as a food medium. With regard to the possibility of phagocytosis, embryonic cells of the parasite were never observed to be under phagocytic attack, but several of the sections showed blood cells of the host which had become applied to the serosa. This was particularly obvious in the three-day embryos in *Macrosiphum euphorbiae*, and the reasons for discounting intracellular phagocytosis of the parasite serosa as a means of defence have been given under that section.

To distinguish between explanations (ii) and (iii) is a difficult matter. It has

been explained that the main growth in size of the embryo occurs from about the 24-hour stage onwards, *i.e.*, after the formation of the serosa. It is presumably at this point, therefore, that any deficiencies in the host as a nutritional medium would become apparent and it may be significant that the development of the parasite in the false hosts does not normally proceed beyond this point. Conversely, it could be argued that it is at precisely this stage that the developing parasite comes to lie just outside the nervous tissue of the host where it would become exposed to active secretions of the host, if such are produced.

It is necessary, therefore, to examine the histological details of the degeneration processes in order to decide between these opposing hypotheses. Despite the variety of appearances exhibited by degenerating embryos, it would be generally true to say that these processes consist of vacuolisation and shrinkage. It is true that the serosa becomes vacuolated in the true host during the formation of the serosal masses (figs. 29a-d) but in this case vacuolisation is less drastic; it occurs not at one day after attack but at three and is associated with hypertrophy of the serosa and not with its flattening and disappearance. The shrinkage of the embryo cells is well illustrated in the sections of *M. euphorbiae* (figs. 17-21) where the cells can be seen to be becoming smaller and more darkly staining in sections at successively later stages. The progressively darker appearance of these cells is attributed to the fact that the cytoplasm is becoming withdrawn from them so that all that remains is the intensely staining nuclear material which, at this stage, occupies most of the cell and appears to be disorganised in that fragments of it lie around the periphery of the cell. The withdrawal of yet more cytoplasmic material results in further contraction of the cells to minute pieces of nuclear material measuring only 2μ in diameter. A few days later these too have disappeared. Similar changes occur in those parasitic embryos in *Myzus persicae* and *A. solani* which develop vigorously up to the 24-hour stage, but it is interpreted that in those individuals where degeneration sets in at an earlier stage of development, vacuolisation and shrinkage leads to the formation of 'speckly' masses such as are shown in figs. 19c, 22d and 23.

Now cells which greatly resemble those of the degenerating parasite embryo cells have been seen elsewhere on the slides of sectioned aphid material. Firstly, in the sections of the development of the parasite in the true host, *N. ribis-nigri*, at the three-day and later stages, degenerating aphid embryos were present, and close examination reveals that their cell structure (fig. 8b) is similar to that of degenerating parasite embryos from false hosts, the larger cells ($3-4\mu$ in diameter) having a small amount of vacuolated cytoplasm and a comparatively high proportion of irregularly arranged nuclear material, and the smaller (approximately 2μ in diameter) appearing to consist of nuclear material only. The appearance of degenerating aphid embryo and parasite embryo cells is, therefore, very similar. Spencer (1926), in describing the changes in aphid embryo cells resulting from successful parasite attack, expresses the belief that these changes are brought about by a digestive or cytolytic enzyme secreted by the parasite.

Secondly, the 36-hour stages in *M. persicae* which had to be abandoned as the aphids were already parasitised before the experiments began, proved valuable in that they contained large larvae of some other parasite, some of which had been actively feeding on the tissues of their hosts and which contained host cells in their guts. These cells (fig. 30) consisted of intensely staining, fragmented nuclear material together with a small amount of vacuolated cytoplasm and they greatly resembled the cells of degenerating parasite embryos. There can be little doubt, in this instance, that their appearance was the result of the action of digestive processes.

There is, therefore, ground for supposing that, in *Macrosiphum euphorbiae*, *Myzus persicae* and *A. solani*, the parasite succumbs to chemical agents secreted by its host, *i.e.*, that the immunity is of humoral nature. But it is possible that,

in these species, there are additional factors which restrict the growth of the parasite. In the species *Myzus persicae* and *A. solani* for example, the development of the parasite inexplicably tends to lag behind that in the true host species and it has not been proved that the parasite cells have not been killed by some other method, such as starvation, before they begin to be absorbed.

Summary.

Aphids belonging to the species *Nasonovia ribis-nigri* (Mosley), *Aulacorthum circumflexum* (Buckt.), *Macrosiphum euphorbiae* (Thos.), *Myzus persicae* (Sulz.) and *A. solani* (Kalt.) were exposed to attack by the parasite, *Monoctonus paludum* Marshall, and were sectioned and dissected at various time-intervals after attack in order to observe the developmental processes of the parasite.

The egg was always laid in the posterior mass of fused ventral nerve ganglia in the thorax of the aphid host.

The development of the parasite in *N. ribis-nigri*, its normal host on lettuce, is described.

In the species *A. circumflexum* the development of the parasite was arrested after approximately 24 hours by the secretion around it of a thin brown capsule formed by certain of the host's blood cells.

In the other three species, *Macrosiphum euphorbiae*, *Myzus persicae* and *A. solani*, no capsule formation occurred. The development of the parasite embryos was rather variable in these but it was common for parasites in *Macrosiphum euphorbiae* to reach the stage normal for 24-hour embryos and for parasites in *Myzus persicae* and *A. solani* to reach stages rather less advanced than this before degenerative changes set in.

The degenerative changes in the parasite embryos are described for each species of aphid and the nature of these changes is discussed.

Acknowledgements.

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STUDIES OF THE SAMPLING OF *GLOSSINA PALLIDIPE* AUST.

I.—THE NUMBERS CAUGHT DAILY ON CATTLE, IN MORRIS TRAPS AND ON A FLY-ROUND.

By I. M. SMITH * and B. D. RENNISON †

*East African Trypanosomiasis Research Organization,
Tororo, Uganda.*

I. G.

The increasing use of chemoprophylactic drugs against bovine trypanosomiasis has brought to light problems in their application, one of the more important of which appears to be trypanosome challenge. One definition of this term and a summary of the many factors possibly involved was given by Smith & Rennison (1960). Its importance was recognised (Anon., 1955; Whiteside, 1960), and it was observed that, in the areas in which this work was done, the greater the density of tsetse, the more frequently a prophylactic drug had to be administered to obtain continuous and apparently complete protection. To establish estimates of the density of tsetse (as well as of many other animals) the now classical work of Jackson (1933, 1937, 1941, 1944, 1949) is available, using essentially the fly-round technique (Potts, 1930) as developed by several East African workers (Buxton, 1955). As will be discussed later, bias occurs in fly-round data and it was thought that a comparison of several methods of catching tsetse in which allowance was made for some of the known variables involved might yield information of value; the lack of such studies was commented upon at length by Buxton (1955). Accordingly, experiments were carried out, in two successive years, in which catches of tsetse off cattle and in traps were compared with catches made concurrently on standard fly-rounds.

Materials and methods.*Locality.*

The experiments were carried out in Uganda, near Lugala (lat. 0° 12'N., long. 33° 57'E.), in the Busoga fly-belt lying along the north-eastern shore of Lake Victoria. The area is part of that in which occurred the great sleeping-sickness epidemic at the beginning of this century (Scott, 1939). The experimental area was part of a drainage line a few hundred yards from the lake littoral (fig. 1). Pilson (see Rennison, 1960) described the vegetation of the drainage line as seasonally waterlogged grassland bounded on two sides by secondary dry forest. Although the area is infested chiefly by *Glossina pallidipes* Aust., small numbers of *G. brevipalpis* Newst. and *G. palpalis fuscipes* Newst. are found.

Methods of catching.

Cattle.—Two-year-old, small, shorthorned East African Zebu oxen were used.

Fly-round.—Before, during and after each experiment, catches were made between 0800 and 0930 hr. (East African Standard Time) two or three times weekly by the standard methods (Buxton, 1955) along a path 3,800 yd. long and divided into 50-yd. sectors (fig. 1). During the second experiment, the 1,000-yd.

* Now at Trinity College, Dublin.

† Now with Department of Agriculture, Kampala, Uganda.

portion of the fly-round along which the experimental methods of catching were used was traversed seven times daily in each direction between 0800 and 1830 hr.

Traps.—Harris (1932) and Jack (1941) found that examples of *G. pallidipes*, including a high proportion of females, readily entered a Harris trap, but this trap is more cumbersome than the one designed to resemble a small ruminant with which Morris & Morris (1949) caught many examples of *G. tachinoides* Westw.

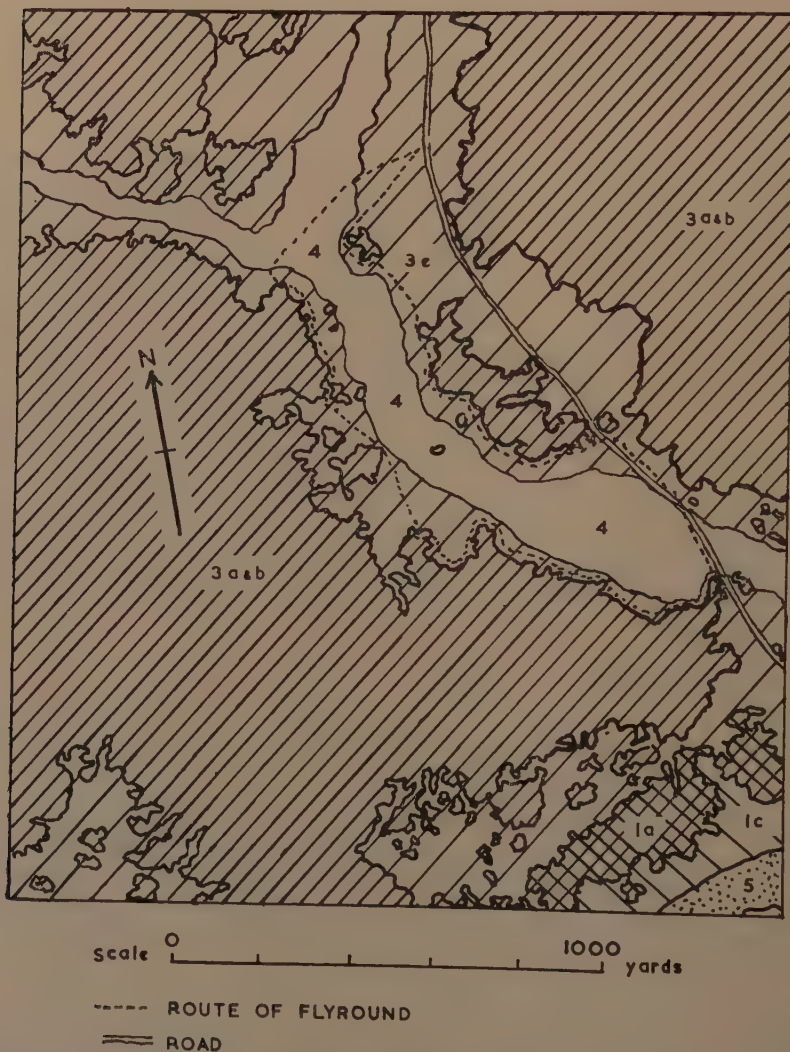


Fig. 1.—A vegetation map* of the experimental area at Lugala, Uganda, on the north-eastern shore of Lake Victoria, showing the fly-round along the south-western part of which the experiments were sited. Key to vegetation phases (after Pilon (see Rennison, 1960)): 1a and c, sub-phases of lake-shore vegetation; 3a, b and c, sub-phases of secondary dry forest; 4, seasonally waterlogged grassland; 5, hygrophilous communities.

* Traced from an aerial photograph, and reproduced by kind permission of the Director of Lands and Survey Department, Entebbe, Uganda.

and *G. palpalis palpalis* (R.-D.), and which was shown by Morris (in Glasgow, 1956) to take large numbers of *G. pallidipes*. Our traps were of the latter type, with the cages shortened by six inches, following the modification of Morris (1960). The hessian covering the body of the traps was either the natural light brown or painted black, and for each experiment new hessian was fitted.

Recordings.

Only flies that actually alighted on the oxen were caught; they were then killed, recorded and retained, the sex of each being noted. Wet- and dry-bulb readings were made every $1\frac{1}{2}$ hr. from 0800 to 1830 hr. Rainfall was measured in a gauge half a mile from the experimental area.

Plans of experiments.

The first experiment was carried out in 1957 and an 8×8 Latin-square design was employed, involving randomisation of the attractants, which were variously coloured oxen and traps. Sites were taken at random from posts demarcating the first 40 sectors of the fly-round (fig. 1), starting at the extreme south-westerly point. The attractants were a red, a white and a black ox, a brown trap (that is, covered with the natural-coloured hessian), a black trap (that is, covered with hessian painted black), pairs of brown and of black traps, and a black and a brown trap as a pair. The oxen were tethered by ropes permitting movement over an area of about 10 yards' diameter, and two fly-boys were delegated to each animal to make the catches. The cattle were not allowed to lie down. The traps were placed with their long axes parallel to the thicket edge and about one yard from it; paired traps were aligned end to end, one foot apart. They were tended by one of the fly-boys from the nearest ox. Flies were caught from 0800 to 1830 hr. daily from 29th March to 5th April 1957, inclusive (first replicate), and, after a four-day interval, from 10th to 17th April (second replicate). The long rains began in mid-March, and a total of 10.4 in. fell during the experiment.

A second experiment, using sites taken at random from amongst the first 20 sectors of the fly-round, was carried out from 13th to 21st March 1958, before the onset of the long rains, and was based on a 9×9 graeco-Latin square design to include fly-boys, sites, days and attractants. The attractants were a red, a white, a black and a red-and-white spotted ox, and five single traps covered with natural hessian. The cattle were tethered and the traps placed as in Expt. 1. Fly-boys not catching from the cattle withdrew to a central point about half a mile from the traps and when emptying the latter approached directly from across the grassland. During the nine days, 2.9 in. of rain fell, nearly all of it in 24 hours, and associated with a violent thunderstorm.

Treatment of data.

The numbers of *G. brevipalpis* and *G. f. fuscipes* captured (less than 0.05% of the total) and of teneral individuals of *G. pallidipes* were ignored in the compilation of the data (the teneral flies will be dealt with subsequently). Hereafter, the term 'flies' will refer to non-teneral adults of *G. pallidipes*. Analyses were based on the values of $\log(n+1)$, where n represented the number of adult males or females of *G. pallidipes* caught each day on each attractant; this device of Williams (1937) was used because, for each attractant, the standard deviation of the catches was proportional to their mean value. The numbers in two traps catching as a pair were summed before transformation when the pair was regarded as one attractant but were transformed separately when mean values of brown or black traps were considered.

palpalis

TABLE I.

The numbers of non-teneral examples of *G. pallidipes* caught on oxen and in Morris traps in Expt. 1, first replicate, at Lugala, Uganda (0800-1830 hr., 29.iii-5.iv.57).

Day	Sites																Totals	
	1 ♂♂ ♀♀	2 ♂♂ ♀♀	3 ♂♂ ♀♀	4 ♂♂ ♀♀	5 ♂♂ ♀♀	6 ♂♂ ♀♀	7 ♂♂ ♀♀	8 ♂♂ ♀♀	Days ♂♂ ♀♀	Attractants ♂♂ ♀♀								
1	19 A	54 C	18 58	116 G	54 F	34 76	115 H	68 D	14 7	E	9 20	115 B	70	440	407	218 A	725	
2	85 B	82 A	21 59	37 E	123 D	10 20	34 F	68 G	79 52	C	11 35	185 H	120	462	559	846 B	493	
3	10 D	31 E	16 49	156 H	145 A	4 40	108 B	62 F	20 35	G	252 136	14 C	19	580	517	127 C	405	
4	16 F	37 G	58 56	10 C	18 B	72 55	8 E	28 H	163 100	D	24 106	38 A	79	389	479	172 D	475	
5	114 H	32 B	46 22	23 D	89 C	15 44	165 G	109 A	21 70	F	29 90	32 E	106	445	562	178 E	625	
6	36 E	188 F	25 94	47 A	207 H	290 153	30 D	135 C	10 60	B	175 74	254 G	140	867	1051	379 F	885	
7	40 C	82 D	41 65	148 B	75 G	287 159	43 A	121 E	31 49	H	460 214	185 F	282	1235	1047	1459 G	913	
8	248 G	207 H	146 116	36 F	203 E	9 62	9 C	89 B	97 53	A	25 95	20 D	22	590	847	1629 H	948	
Site totals	568	713	371	519	573	914	721	609	512	680	435	426	985	770	843	838	5008	5469

B, G, H=White, red and black oxen, respectively.

C=Trap with black-painted hessian.

D=Pair of traps, each with black-painted hessian.

A=Pair of traps, one with black-painted and the other with natural-coloured (brown) hessian.

E=Trap with natural-coloured (brown) hessian.

F=Pair of traps, each with natural-coloured (brown) hessian.

Personnel.

Fly-boys were supervised by an African laboratory assistant who kept the psychrometer and rainfall records. Over-all control was maintained by the daily presence of one or other author, who made check counts of the captured flies.

Results.

The numbers of *G. pallidipes* caught in the two replicates of Expt. 1 and in Expt. 2 are shown in Tables I, II and III, respectively; appended to each Table is an analysis (Tables IA–IIIA) from which general indications can be obtained.

TABLE IA.

Analysis of data (transformed) from Table I.

Source of variance						d.f.	Mean square	F
Days (D)	7	0.4021	5.00***
Sites (S)	7	0.1056	1.31
Attractants (T)	7	1.2464	15.48***
White v. coloured oxen	1	0.6208	7.71**
Red v. black ox	1	0.0105	0.13
Pair brown traps v. single brown trap	1	0.3200	3.98
Pair black traps v. single brown trap	1	0.0190	0.23
Black v. brown traps	1	0.6848	8.51**
Mixed pair v. black plus brown traps	1	0.1304	1.62
Oxen v. traps	1	6.9396	86.21***
Error	42	0.0805	—
Sexes (X)	1	1.2423	94.83***
D × X	7	0.0373	2.85*
S × X	7	0.0504	3.84**
T × X	7	0.4902	37.42***
Between oxen	2	0.0019	0.14
Between traps	4	0.0271	2.06
Oxen v. traps	1	3.3192	253.37***
Error	42	0.0131	—

* $P \leq 0.05$. ** $P \leq 0.01$. *** $P \leq 0.001$.

Days.

Very different numbers of flies were caught on different days ($P < 0.001$ in each of the three analyses) and the data indicate the intricate pattern of behaviour in the insect in response to climatic and other stimuli.

On the fourth day of the second replicate of Expt. 1, and on the fifth day of Expt. 2, the traps caught very few flies. Both days were cloudy though it rained only on the former. Our trap results were obviously affected by these apparently adverse conditions. Thus the attractive power of Morris traps clearly depends in part at least on a climatic factor governed by sun intensity.

TABLE II.

The number of non-feneral examples of *G. pallidipes* caught on oxen and in Morris traps in Expt. 1, second series, at Lugala, Uganda (0800-1830 hr., 10-17.iv.57).

replicate

Days	Sites																Totals	
	1	2	3	4	5	6	7	8	Days								♂♂	♀♀
1	♂♂ 34 E	♂♂ 98 F	♂♂ 43 A	♂♂ 10 D	♂♂ 277 G	♂♂ 32 C	♂♂ 187 B	♂♂ 332 H	♀♀ 211 E	♀♀ 230 F	♀♀ 163 A	♀♀ 37 D	♀♀ 200 G	♀♀ 48 C	♀♀ 123 B	♀♀ 205 H	♂♂ 1013	♀♀ 1217
2	♂♂ 187 B	♂♂ 249 G	♂♂ 51 C	♂♂ 166 H	♂♂ 63 A	♂♂ 19 D	♂♂ 14 E	♂♂ 63 F	♀♀ 109 B	♀♀ 127 G	♀♀ 71 C	♀♀ 84 H	♀♀ 136 A	♀♀ 24 D	♀♀ 90 E	♀♀ 105 F	♂♂ 812	♀♀ 746
3	♂♂ 8 C	♂♂ 78 B	♂♂ 167 G	♂♂ 14 E	♂♂ 10 D	♂♂ 69 H	♂♂ 25 F	♂♂ 55 A	♀♀ 49 C	♀♀ 61 B	♀♀ 140 G	♀♀ 29 E	♀♀ 32 D	♀♀ 72 H	♀♀ 66 F	♀♀ 59 A	♂♂ 426	♀♀ 508
4	♂♂ 4 A	♂♂ 3 D	♂♂ 86 H	♂♂ 89 B	♂♂ 0 C	♂♂ 1 F	♂♂ 91 G	♂♂ 1 E	♀♀ 1 A	♀♀ 2 D	♀♀ 34 H	♀♀ 59 B	♀♀ 5 C	♀♀ 0 F	♀♀ 45 G	♀♀ 2 E	♂♂ 275	♀♀ 148
5	♂♂ 145 H	♂♂ 37 C	♂♂ 35 D	♂♂ 146 G	♂♂ 9 F	♂♂ 5 E	♂♂ 34 A	♂♂ 249 B	♀♀ 104 H	♀♀ 72 C	♀♀ 99 D	♀♀ 139 G	♀♀ 45 F	♀♀ 28 E	♀♀ 81 A	♀♀ 177 B	♂♂ 660	♀♀ 745
6	♂♂ 169 G	♂♂ 97 A	♂♂ 24 E	♂♂ 12 F	♂♂ 96 H	♂♂ 134 B	♂♂ 12 D	♂♂ 11 C	♀♀ 100 G	♀♀ 177 A	♀♀ 131 E	♀♀ 57 F	♀♀ 116 H	♀♀ 122 B	♀♀ 48 D	♀♀ 42 C	♂♂ 555	♀♀ 793
7	♂♂ 64 F	♂♂ 272 H	♂♂ 306 B	♂♂ 33 A	♂♂ 43 E	♂♂ 203 G	♂♂ 26 C	♂♂ 36 D	♀♀ 142 F	♀♀ 213 H	♀♀ 179 B	♀♀ 66 A	♀♀ 106 E	♀♀ 203 G	♀♀ 38 C	♀♀ 34 D	♂♂ 1234	♀♀ 981
8	♂♂ 40 D	♂♂ 25 E	♂♂ 25 F	♂♂ 11 C	♂♂ 171 B	♂♂ 35 A	♂♂ 148 H	♂♂ 148 G	♀♀ 41 D	♀♀ 87 E	♀♀ 79 F	♀♀ 33 C	♀♀ 109 B	♀♀ 49 A	♀♀ 94 H	♀♀ 123 G	♂♂ 603	♀♀ 615
Site totals	651	757	859	969	737	896	481	504	669	749	546	537	585	895	747		5578	5753

B, G, H = White, red and black oxen, respectively.

C = Trap with black-painted hessian.

D = Pair of traps, each with black-painted hessian.

A = Pair of traps, one with black-painted and the other with natural-coloured (brown) hessian.

E = Trap with natural-coloured (brown) hessian.

F = Pair of traps each with natural-coloured (brown) hessian.

Sites.

Other workers, *e.g.*, Harris (1932) and Morris & Morris (1949), stressed the care necessary in siting traps. However, there was no significant variation between the various positions used during the experiments as regards numbers of flies caught (Tables IA, IIA, IIIA); and this suggests that great skill is not essential in all situations in siting traps, although it should be remembered that the conditions and the vegetation in which we worked were comparatively uniform. Morris & Morris (1949) regarded as significant two- and five-fold differences in trap catches made only a few yards apart, but in our experiments the over-all effect of the site was not significant, even though more than three times as many flies were caught at one site as another.

TABLE IIA.

Analysis of data (transformed) from Table II.

Source of variance	d.f.	Mean square	F
Days (D)	7	1.8686	17.50***
Sites (S)	7	0.2044	1.91
Attractants (T)	7	2.0036	18.76***
Oxen	2	0.0448	0.42
Pair brown traps <i>v.</i> single brown trap ..	1	0.0561	0.52
Pair black traps <i>v.</i> single black trap ..	1	0.0036	0.03
Black <i>v.</i> brown traps	1	0.2678	2.51
Mixed pair <i>v.</i> black plus brown traps ..	1	0.7125	6.67*
Oxen <i>v.</i> traps	1	12.8953	120.74***
Error	42	0.1068	—
Sexes (X)	1	0.7859	42.71***
D × X	7	0.0714	3.88**
S × X	7	0.0309	1.68
T × X	7	0.3314	18.01***
Oxen	2	0.0008	0.04
Pair brown traps <i>v.</i> single brown trap ..	1	0.0722	3.92
Pair black traps <i>v.</i> single black trap ..	1	0.0512	2.78
Black <i>v.</i> brown traps	1	0.0564	3.06
Mixed pair <i>v.</i> black plus brown traps ..	1	0.1148	6.24*
Oxen <i>v.</i> traps	1	2.0234	109.97***
Error	42	0.0184	—

* $P \leq 0.05$. ** $P \leq 0.01$. *** $P \leq 0.001$.*Attractants.*

Significantly more flies were caught on the cattle than in the traps. In Expt. 1, fewer flies were caught on the white ox than on the coloured ones in the first replicate, but not in the second. For the latter the fly-boy pairs changed oxen, the first pair going from the white animal to the black, the second from the red to the white and the third from the black to the red. Thus, there possibly occurred a 'fly-boy effect' and in consequence no conclusions could be reached on the attractiveness to the fly of oxen of different colour. In Expt. 2 (Tables III, IIIA), there was no significant variation between catchers. Fewer flies were attracted to the white ox than to the other oxen, of which the red ox

TABLE III.

The numbers of non-teneral examples of *G. pallidipes* caught on oxen and in Morris traps in Expt. 2 at Lugala, Uganda (0800-1830 hr., 18-21.iii.58).

Days	Sites										Totals	
	1 ♂♂ ♀♀	2 ♂♂ ♀♀	3 ♂♂ ♀♀	4 ♂♂ ♀♀	5 ♂♂ ♀♀	6 ♂♂ ♀♀	7 ♂♂ ♀♀	8 ♂♂ ♀♀	9 ♂♂ ♀♀	Days ♂♂ ♀♀	Attractants ♂♂ ♀♀	Catchers ♂♂ ♀♀
1	E.8 102 69	B.5 6 20	I.3 4 8	A.4 206 109	F.9 36 52	G.1 3 16	D.7 26 13	H.2 4 15	C.6 30 25	417 327	A 1147 844	1 330 223
2	A.1 147 103	G.7 6 18	E.5 133 70	I.9 1 1	B.2 8 11	F.6 15 21	C.3 61 53	D.4 44 14	H.8 3 7	418 298	B 61 116	2 547 565
3	H.9 2 5	E.6 72 34	C.1 69 50	D.5 46 25	I.7 4 5	A.2 276 350	G.8 2 27	B.3 7 10	F.4 3 15	481 521	C 694 417	3 270 157
4	D.2 102 41	A.8 101 52	H.6 1 1	C.7 86 32	E.3 75 34	I.4 2 4	F.1 2 8	G.5 4 1	B.9 2 3	375 176	D 422 155	4 491 281
5	F.5 0 1	C.2 46 32	G.9 0 0	B.1 0 2	D.6 29 10	H.7 3 5	E.4 91 35	I.8 1 0	A.3 58 18	228 103	E 748 360	5 395 237
6	B.7 1 4	H.4 4 7	F.2 3 4	G.6 4 8	C.8 70 60	D.3 56 11	A.9 101 65	E.1 72 32	I.5 2 5	313 196	F 69 132	6 227 158
7	G.3 3 9	D.9 45 14	B.4 19 25	F.8 2 14	H.1 1 5	C.5 82 31	I.2 15 56	A.6 62 31	E.7 58 20	287 205	G 28 104	7 262 149
8	C.4 119 57	I.1 1 1	D.8 39 16	H.3 0 2	A.5 120 69	E.9 55 20	B.6 9 18	F.7 2 5	G.2 3 10	348 198	H 20 62	8 329 268
9	I.6 5 10	F.3 6 12	A.7 76 47	E.2 90 46	G.4 3 15	B.8 9 23	H.5 2 15	C.9 131 77	D.1 35 11	357 256	I 35 90	9 373 237
Site totals	481 299	287 190	344 221	435 239	346 261	501 481	309 290	327 185	194 114	3224 2280	3224 2280	3224 2280

A, C, D, E=Red, red-and-white spotted, white and black oxen, respectively. B, F, G, H & I=Traps covered with natural-coloured hessian.
1-9=Catchers.

was the most attractive. This result conflicts with that of Moggridge (1936) who indicated (though without excluding possible differences between fly-boys) that fewer individuals of *G. swynnertoni* Aust. came to red than white or black animals.

During Expt. 1, traps of different colours caught dissimilar numbers of flies. Those covered with natural-coloured hessian usually took more flies than the black traps, but the differences were significant in the first replicate only; much

TABLE IIIA.

Analysis of data (transformed) from Table III.

Source of variance						d.f.	Mean square	F
Days (D)	8	0.5168	4.49***
Sites (S)	8	0.1880	1.63
Attractants (T)	8	4.9894	43.39***
White ox v. coloured oxen	1	1.9342	16.82***
Red ox v. black ox plus mottled ox	1	0.4511	3.92
Black v. mottled ox	1	0.0011	0.01
Traps	4	0.2408	2.09
Oxen v. traps	1	36.5659	317.96***
Catchers (C)	8	0.2116	1.84
Error	48	0.1150	—
Sexes (X)	1	0.0444	2.88
D × X	8	0.0423	2.75*
S × X	8	0.0421	2.73*
T × X	8	0.4800	31.17***
White ox v. coloured ox	1	0.0988	6.42*
Red ox v. black ox plus mottled ox	1	0.0065	0.42
Black v. mottled ox	1	0.0361	2.34
Traps	4	0.0349	2.27
Oxen v. traps	1	3.5588	231.09***
C × X	8	0.0222	1.44
Error	48	0.0154	—

* $P < 0.05$. ** $P < 0.01$. *** $P < 0.001$.

of this variation is attributable to high catches in the paired brown traps on the eighth day. In Expt. 2, when all the traps were of the same colour, no significant variations occurred between the numbers of flies they caught.

The superiority of brown traps in Experiment 1 does not accord with earlier findings (Simpson, 1911; Swynnerton, 1933, 1936; Lloyd, 1935) that tsetse were attracted to dark surfaces in greater numbers, from which observation has arisen the practice of carrying dark screens on fly-rounds when catching species of low availability (for summary, see Buxton, 1955). Jack (1941) and Morris (in Willett, 1956) also found black coverings markedly better than brown when trapping *G. pallidipes*. Morris (in Willett, 1956) used hessian dyed black, and this material may have been better than painted hessian either because the paint repelled or because it produced a physical alteration in attractiveness arising, perhaps, from a subtle alteration in the local microclimate. On the other hand,

were darker colours more attractive to *G. pallidipes*, the black ox should have attracted the greater number of flies; this was not so, and the finding suggests to us that bait-animals evoke a specific response in the insect which differs from those elicited by other attractants, a view also held by E. Bursell (The behaviour of tsetse flies (*G. swynnertoni*) in relation to problems of sampling *).

In Experiment 1, the paired traps of either colour failed to catch markedly more flies than the single traps of the same colour. This implies that the two members of a pair of almost contiguous traps sample the same area. A similar effect may have been a partial cause of the large differences recorded by Morris & Morris (1949) between places with pairs of unequally spaced traps.

TABLE IV.

Catches of *G. pallidipes* on oxen, in Morris traps and on fly-rounds in experiments at Lugala, Uganda, expressed as Williams' means* of daily catches of non-teneral flies (data of Tables I, II, III & VI).

Exp. 1 (1957)	M _w	
	♂♂	♀♀
Brown trap (29.iii-17.iv) ..	14	42
Oxen (29.iii-17.iv) ..	140	84
Fly-round (16.iii-1.v) ..	11	7
Exp. 2 (1958)	M _w	
	♂♂	♀♀
Brown trap (13-21.iii) ..	5	11
Oxen (13-21.iii) ..	84	49
Fly-round (27.ii-4.iv) ..	13	7

* The Williams' mean, M_w (Haddow, 1960, Appendix), is defined by

$$\log(M_w+1) = \frac{\sum \log(n_i+1)}{N}$$

where n_1, n_2, \dots represent the actual values of a series of N observations.

Sexes.

Traps took a higher proportion of females than did the cattle ($P < 0.001$), a finding which suggests the two methods of catching may attract tsetse for different reasons. There were no significant differences between the sex ratios of the catches on different coloured oxen in Expt. 1, but proportionally fewer females were caught on the white ox in Expt. 2 ($P < 0.05$). Black traps and brown traps took similar proportions of males and females. The sex ratio of the samples taken by any given method varied little from experiment to experiment (Table V).

A significant interaction of sex \times days in both experiments and of sex \times site in the first replicate of Expt. 1 and in Expt. 2 demonstrates that the sexes were not equally influenced by the factors controlling activity and availability, and thus strengthens the view, long held by many workers, that the behaviour pattern of the sexes differs.

* To appear in *Proc. R. ent. Soc. Lond.* (A) 36.

Fly-round.

The catches made on the fly-round (fig. 1) are not directly comparable with the ox or trap catches. Nevertheless, useful information can be inferred from a consideration of them.

TABLE V.

The sex ratios of non-teneral examples of *G. pallidipes* caught in Expts. 1 and 2.

	Method of catching	No. of flies		Ratio
		♂♂	♀♀	
Expt. 1	Brown traps	1341	3916	1/2.9
	Black traps	884	2013	1/2.3
	Oxen	8344	5292	1/0.6
	Fly-round (16.iii-1.v.57)	213	123	1/0.6
Expt. 2	Brown traps	213	504	1/2.4
	Oxen	3011	1774	1/0.6
	Fly-round	604	97	1/0.2
	Fly-round (27.ii-4.iv.58)	299	159	1/0.5

In Table VIA are given the numbers of non-teneral examples of *G. pallidipes* caught on successive traverses of the fly-round, beginning two weeks before and

TABLE VI.

Catches of non-teneral examples of *G. pallidipes* made on fly-rounds in the experimental area at Lugala, Uganda.

A. Standard catches (0800-0930 hr.) on whole fly-round (3,800 yd.).

Numbers caught on successive traverses from two weeks before until two weeks after the experiment.

Expt. 1 (1957)			Expt. 2 (1958)		
Date	♂♂	♀♀	Date	♂♂	♀♀
16.iii	3	2	27.ii	26	12
19.iii	7	6	1.iii	22	6
21.iii	10	10	3.iii	8	2
23.iii	8	7	5.iii	18	5
26.iii	41	17	7.iii	10	4
28.iii	16	11	9.iii	10	0
30.iii	16	2	11.iii	35	14
2.iv	9	13	13.iii	30	11
5.iv	8	9	15.iii	17	13
9.iv	7	11	17.iii	12	14
12.iv	11	6	19.iii	10	15
16.iv	9	7	21.iii	26	6
26.iv	7	5	23.iii	21	3
29.iv	52	14	25.iii	10	15
1.v	9	4	27.iii	1	3
			29.iii	10	11
			2.iv	27	21
			4.iv	6	1
Totals ..	213	124	Totals ..	299	159
Mean A.D. ..	37.4		Mean A.D. ..	43.7	

A.D. = Apparent density (non-teneral (N.T.) males per 10,000 yd.).

ending two weeks after each experiment; Table VIB gives the numbers taken on the 1,000-yd. section along which the cattle and traps were distributed in the second experiment. The mean apparent density (Nash, 1933; Buxton, 1955) was higher in the second experiment, but not significantly so, and the observation of Jackson (1944) that fly-round catches of *G. morsitans* Westw. gave a reasonable measure of the true density of the population would suggest, if true of *G. pallidipes* at Lugala, that the density of the fly populations did not differ in the two

TABLE VI—continued.

B. Special catches on 1,000-yd. experimental section (1958).

Date	Mean numbers caught on seven traverses, 0800–1830 hr.		Numbers caught 0800 to 0930 hr. only	
	♂♂	♀♀	♂♂	♀♀
13.iii ..	9.9	3.7	11	10
14.iii ..	5.6	1.3	10	4
15.iii ..	3.0	0.7	6	4
16.iii ..	11.4	1.7	8	4
17.iii ..	11.7	1.3	4	2
18.iii ..	16.6	1.1	17	1
19.iii ..	5.0	1.1	3	1
20.iii ..	4.7	0.7	6	2
21.iii ..	18.4	2.1	32	2
Totals ..	86.3	13.7	97	30
Mean A.D.	47.9		53.9	

A.D. = Apparent density (non-teneral (N.T.) males per 10,000 yd.)

Analysis of variance (transformed data).

Source	d.f.	Mean sq.	F
Days (D) ..	8	0.0988	3.37
Times of catch (T) ..	1	0.0920	3.13
†Sexes (X) ..	1	2.2600	171.21***
D × T ..	8	0.0293	—
D × X ..	8	0.0676	5.12*
T × X ..	1	0.0822	6.23*
D × T × X ..	8	0.0132	—

† tested against D × T.

* $P \leq 0.05$. ** $P \leq 0.01$. *** $P \leq 0.001$.

experiments. Mean catches off oxen and in brown traps, however, were appreciably lower in Expt. 2 (Table IV). There is no reason to suppose that oxen or traps would provide less reliable measures of tsetse density than fly-rounds, particularly if the catching periods for the two former (0800 to 1830 hr.) included a wider range of the variations in tsetse activity. Possibly, therefore, the population of *G. pallidipes* was smaller during Expt. 2, but the fly-round catches were influenced by differences in the availability of the flies. Comparison of the catches made daily on the experimental section of the fly-round between 0800 and 0930 hr. with the daily means of the catches on seven traverses carried out from 0800 to 1830 hr. (Table VIB) shows that the early-morning catches differed chiefly in containing more females. Thus, in the latter part of the dry season,

the availability of non-teneral males to fly-boys between 0800 and 0930 hr., was reasonably close to the mean availability throughout the day.

The sex ratios in the two series of standard catches on the fly-round (Table IV) differed only slightly from one another and closely approached the ratio from the cattle catches. The lower proportion of females caught on the experimental section of the fly-round during Expt. 2 (see Table VIB) may well have been caused by the presence of the cattle and traps.

Correlations.

No significant serial correlations were found in the data. In Expt. 1, relationships between the catches (fig. 2) were strongly influenced by the wet day (day 4

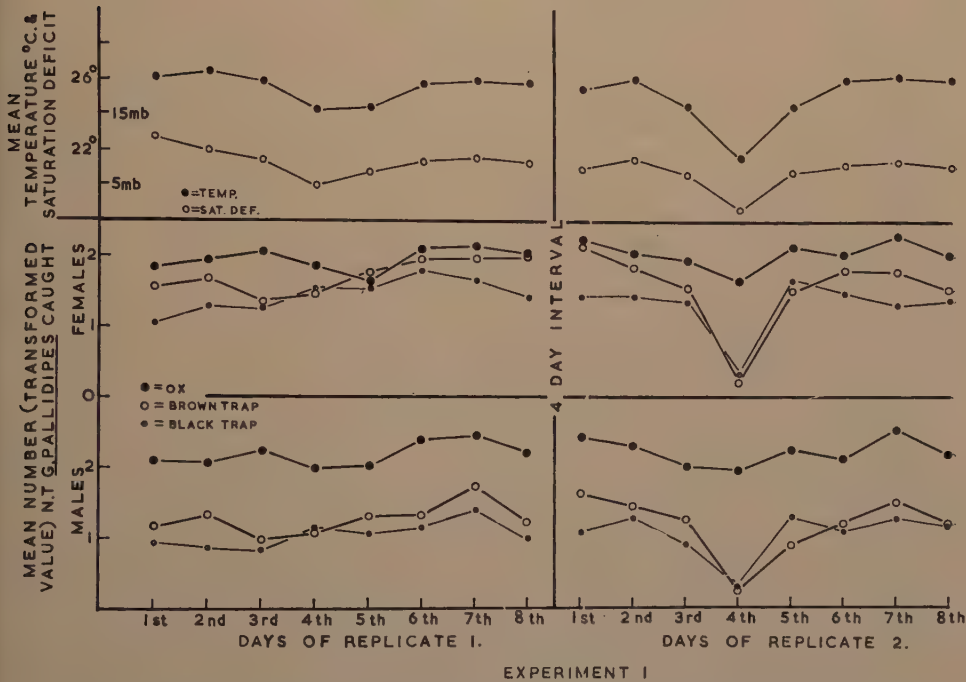


Fig. 2.—The daily means of transformed values of numbers of non-teneral (N.T.) examples of *G. pallidipes* caught by eight Morris traps and by three oxen in Expt. 1 (first and second replicates). The temperatures and saturation deficits shown are mean values of eight measurements made at 1½-hour intervals from 0800 to 1830 hr. daily.

in second replicate, Table II) which so markedly affected trap catches. When these results are omitted, the only significant correlations ($P < 0.05$) involving catches are those, amongst male flies, of mean ox catch with mean brown-trap catch ($r = +0.586$) and mean black-trap catch ($r = +0.599$), and, amongst female flies, of mean saturation deficit and mean black-trap catch ($r = -0.550$). This being so, it seems useless to attempt to relate catches on oxen with those in traps, a view confirmed by the results of Expt. 2, in which the catches with the various attractants were not significantly correlated either amongst themselves or with mean saturation deficit or temperature (fig. 3). Vanderplank (1948) showed that for *G. pallidipes* a threshold occurred at about a temperature of 30°C. and a saturation deficit of 25 mb., above which the bait-ox catch was negatively

correlated with these factors, and below which there was positive correlation. During our two experiments, mean temperatures and saturation deficits were 25.3 and 26.5°C., and 7.7 and 10.6 mb., respectively; thus, in both experiments, temperature and, to a less extent, saturation deficit were close to the levels at which they have little effect on the activity of *G. pallidipes*. Under these conditions, other factors, such as the nutritional state of the flies, the availability of hosts and the state of the vegetation, might be more important in controlling the activity and response of the tsetse to the stimuli provided in the form of oxen, traps and catching parties. In areas, like Lugala, with comparatively equable climate, seasonal fluctuations in density are not so pronounced as in places with greater extremes of heat and drought. Conclusions about the population from samples collected to evaluate such changes can thus be seriously compromised by technical errors.

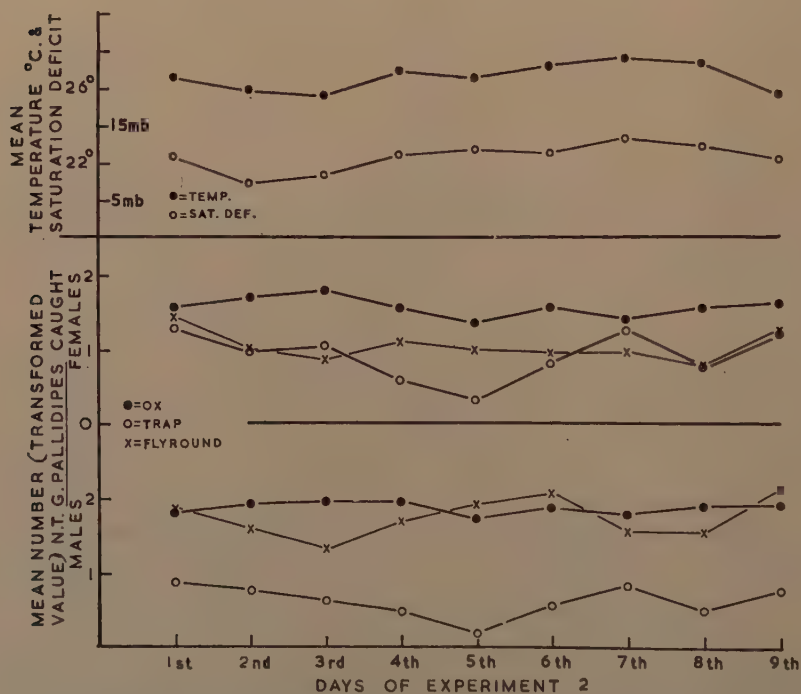


Fig. 3.—The daily means of transformed values of numbers of non-teneral (N.T.) examples of *G. pallidipes* caught on fly-round, by five Morris traps and by four oxen in Expt. 2. Temperatures and saturation deficits determined as in Expt. 1 (fig. 2).

Discussion.

Collecting data on tsetse in the field is easy, interpreting their meaning difficult. We found, for example, light colour more attractive than dark, as far as traps were concerned, thus contradicting many previous observations. In contrast, however, we found a light-coloured animal less attractive than others with darker coats. If this feature can be confirmed for *G. pallidipes*, and if similar distinctions are made by other species, it could be a relevant point in trypanosome challenge where the latter is less than maximum. The oxen we employed were specially chosen for uniformity of conformation and behaviour:

the explanation of their varying attractiveness is obscure and made more so by the fact that in other experiments (unpublished data) white animals were not always less attractive to *G. pallidipes*. No doubt smell also plays a part in attractiveness (Swynnerton, 1936). For these reasons we hesitate to assert that colour preference occurs. Furthermore, it has frequently been assumed that a stationary, or even a recumbent, animal attracts the same sample as an ambulatory one, but it has not been shown that this is so, either numerically or as regards the physiological state of the flies. Cattle under attack by numerous *G. pallidipes* do attempt to lie down so as to protect themselves from attacks concentrated about the legs and, to a less extent, the belly; we kept our animals moving over an area, so as to avoid the possibility of bias in this respect.

As indicated by Expt. 1, the catchers varied among themselves, though all were men of long experience. This variation did not occur in Expt. 2, but in that they were well aware of the implications of the design, and also the work was less arduous. In any event it seems necessary to adopt those methods that permit the isolation of possible variation between individuals in work that demands skill and acuity.

Our finding, that fly-round catches gave similar estimates of density in both the wet season (1957) and the dry season (1958), although the oxen and trap catches were a half, or less, during the latter, stresses the importance of not assuming that fly-round data will give a reliable measure of challenge. Whiteside used as an index of challenge the number of non-teneral tsetse caught per 10,000 yd. of fly-round multiplied by the infection rate of a sample of those caught (Smith & Rennison, 1960). At first sight, the early-morning catch, between 0800 and 0930 hr., which scarcely differed as between oxen and fly-round, appears useful for measuring challenge, at least from *G. pallidipes* at Lugala, but it should be remembered that during the rest of the day the actual challenge would be less than half the estimate. Moreover, the females, which, because of their assumed longer life, are probably the more important sex pathogenically, do not contribute sufficient weight in this index, because of their normally disproportionately low numbers in fly-round catches. Bursell (work quoted on p. 174) adduced further evidence against the use of fly-round data for estimating challenge by showing the flies taken on fly-rounds to be mainly sexually appetitive males, which, for physiological reasons, are not interested in feeding; even if such flies probe, they are much less likely to infect (Fairbairn & Burt, 1946). However, since tsetse feed at fairly regular intervals, those observed as sexually appetitive on a given day will feed at some later time, the effect being to produce a time lag in the estimate of challenge, the figure for which will be reduced by the male death-rate. Unfortunately, tsetse death-rates are themselves very variable. On the other hand, the prophylactic period obtainable with modern drugs is such that, in the field at any rate, there seems no need to estimate challenge on a daily scale.

Although the estimate of challenge as calculated at present may be insensitive, the data recorded in this paper give a preliminary indication that the use of Morris traps, despite the fact that they can sample throughout the day and take a large number of females of *G. pallidipes*, is unlikely to produce a more sensitive measure, as shown by the absence of useful correlation between trap and cattle catches in a situation where temperature and saturation deficit were unlikely to be strongly influencing the catches. Reference has been made to the possibility, suggested by the finding that on some days trap catches were almost *nil* at a time when the cattle continued to attract numerous flies, and by the work of Jack (1941) on the fat reserves of trapped females, that traps may sample only one portion of the population, differing from the portion attracted by cattle; it is hoped to elaborate this point in a subsequent paper. The best estimate of density for the purpose of calculating challenge is direct counts on exposed, grazing cattle, but there are technical difficulties in the method, and sampling vagaries also

arise, such as the problem of whether all male flies on cattle are attempting to feed or are sexually appetitive.

Although the flies move in ambits, reckoned by Jackson (1941) to be of the order of half a mile, the tsetse population is mobile, and consequently the value of the designs we employed can be questioned on several grounds, not least of which is the possibility that a mixture of attractants in a small area may bias the findings, both by generally affecting the experimental area and by producing residual effects at and between the sites. Nevertheless, there are clearly factors in the sampling of tsetse whose effect on the total variation cannot be ignored, and of these, time, place and personnel are important.

Summary.

Two experiments in which *G. pallidipes* Aust. was caught on oxen, in Morris traps and on a fly-round in south-eastern Uganda are described.

Fewer flies were caught in traps than on oxen, but the former took a higher proportion of females. The numbers in traps covered with natural-coloured hessian tended to be greater than in traps on which the hessian was painted black. A white ox attracted fewer flies than darker-coloured oxen, among which a red ox was the most attractive. Variations reflected in day and site effects indicated that the sexes were differentially affected by the factors controlling availability to the oxen and the traps. Fly-round data appeared to give an unsatisfactory estimate of the population density.

The numbers caught by the various methods were, in general, not correlated and this casts doubt on the validity of fly-round or trap data as estimates of the number of *G. pallidipes* likely to attack cattle.

Acknowledgements.

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STUDIES OF THE SAMPLING OF *GLOSSINA PALLIDIPIPES* AUST.—II.
THE DAILY PATTERN OF FLIES CAUGHT ON CATTLE,
IN MORRIS TRAPS AND ON A FLY-ROUND.

By I. M. SMITH * and B. D. RENNISON †

*East African Trypanosomiasis Research Organization,
Tororo, Uganda.*

L. b.

The first paper of this series (Smith & Rennison, 1961) compared the numbers of non-teneral individuals of *Glossina pallidipes* Aust. caught, on successive days in two experiments, by the use of tethered oxen, Morris traps and the standard fly-round technique. Only the total numbers of male and female flies were considered in that paper, although the catches were recorded for every 1½-hour period between 0800 and 1830 hr. (East African Standard Time); the present paper details the daily patterns of the catches.

Materials and methods.

The experimental area at Lugala, Uganda, the design of the experiments, the times they were carried out, the methods of catching and the personnel employed were described in the first paper of this series. For the comparisons described below the numbers of flies caught by each attractant per 1½-hour period (n) were transformed to $\log(n+1)$ (Williams, 1937). When a pair of traps was regarded as a single attractant the sum of the catches in the two traps was transformed, but where mean catches of traps of different colours was under consideration the number of flies taken in each trap of the pair was separately transformed. Wet- and dry-bulb readings were taken with a whirling psychrometer every 1½ hours from 0800 to 1830 hr. during each experiment; moving averages of the temperature and saturation deficit were calculated from them.

Results and discussion.

The daily pattern of catches.

Enough flies were caught in both replicates of Expt. 1 to permit re-analysis of the data to include 1½-hour catching periods. The results already described (Smith & Rennison, 1961) were confirmed. In addition, the data indicate that the flies did not come to the tethered cattle and to the Morris traps randomly throughout the day ($P<0.001$, for both replicates). The periods during which most males were caught differed from those in which most females were caught (fig. 1). A definite difference from day to day in the numbers of flies caught per period of day ($P<0.01$, replicate 1; $P<0.001$, replicate 2) was probably referable to minor variations in weather; the effect was more pronounced in the second replicate, probably because the fourth day was persistently overcast and rainy. The interaction sex \times period \times attractant was not significant in either replicate, *i.e.*, the pattern of catching did not vary for any individual attractant during the experiment; but the difference between the pattern of catches on oxen and that in traps (fig. 1) was confirmed by the significance ($P<0.001$) of the interactions period \times attractant and sex \times attractant.

* Now at Trinity College, Dublin.

† Now with Department of Agriculture, Kampala, Uganda.

The mean counts (fig. 1) of the female flies taken on an ox per period of day in Expt. 1 show that the number coming to the animal during the day was fairly constant, there being fewest shortly after noon and most towards the end of the day. The males behaved in much the same way but numbers were rather greater in the morning, least in the middle of the day and rose markedly in the evening (fig. 1). These results accord with previous findings that the availability of

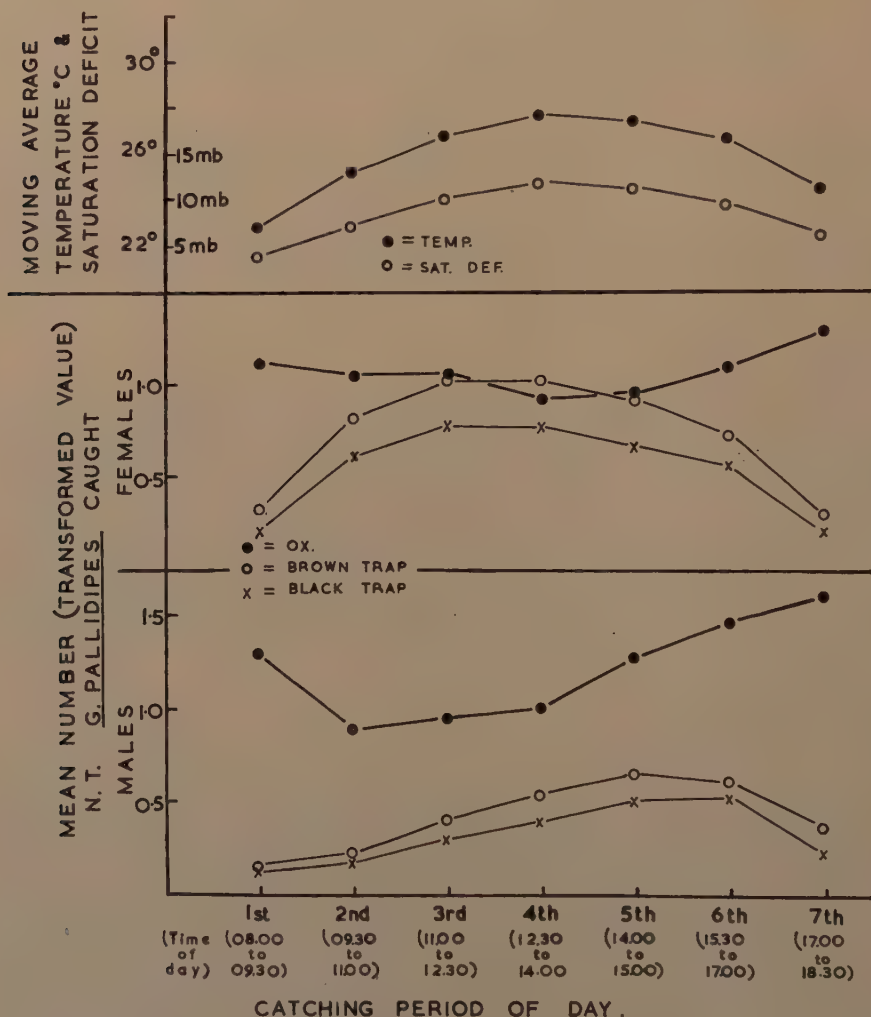


Fig. 1.—Numbers of non-teneral examples of *G. pallidipes* (expressed as mean values of $\log(n+1)$, see p. 183) caught on oxen and in Morris traps during both replicates of Expt. 1 (29.iii–17.iv.57) at Lugala, Uganda.

G. pallidipes is greatest towards the end of day (Croveri, 1919; Curson, 1924; Fuller & Mossop, 1929).

The mean counts for brown traps (those covered with natural-coloured hessian) were noticeably dissimilar from those on the ox (fig. 1). The greatest numbers of females were captured around midday and of males in the early afternoon;

comparatively few of either sex were taken in the morning or evening. Glasgow (1958) reported a similar periodicity in Morris-trap catches of *G. pallidipes*, but Jack (1941) and Swynnerton (1936) gave the impression that the highest catches of *G. pallidipes* occurred between 1500 and 1730 hr. in the various types of trap they used. The catches in black traps (those with the hessian painted black) were essentially similar to those in brown traps, but smaller (fig. 1).

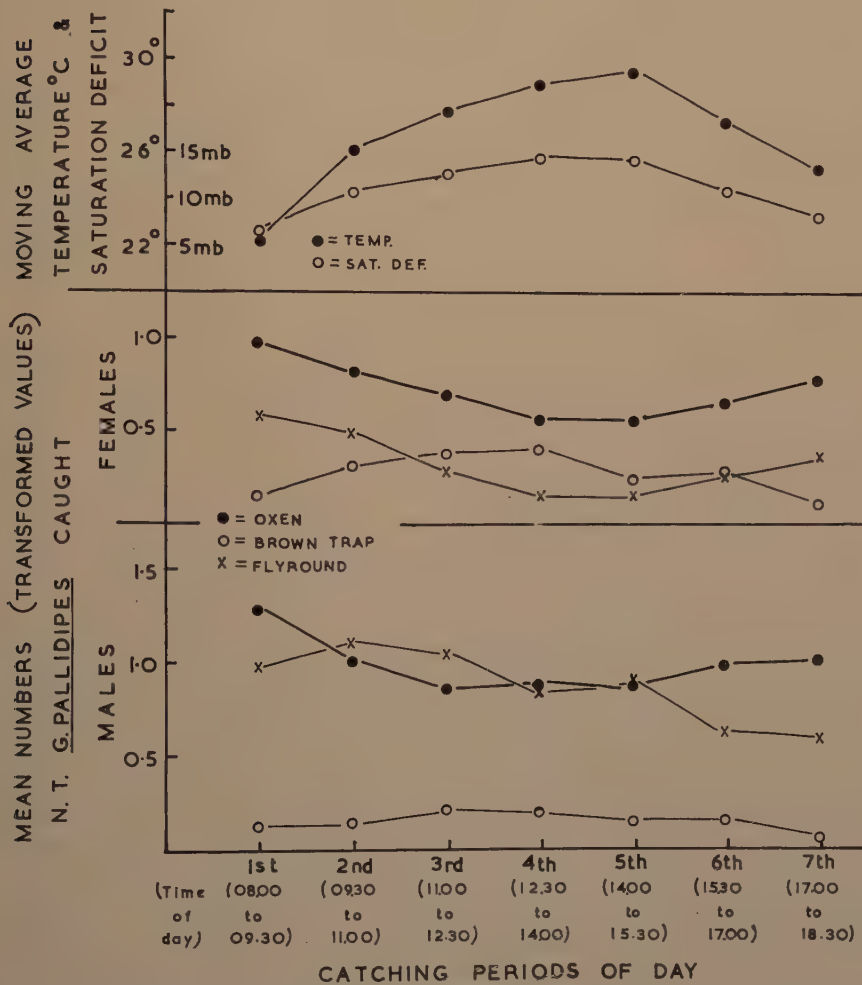


Fig. 2.—Numbers of non-teneral examples of *G. pallidipes* (expressed as mean values of $\log(n+1)$, see p. 183) caught on fly-round, on oxen and in Morris traps during Expt. 2 (13–21.iii.58) at Lugala, Uganda.

The catches in Expt. 2 were very much smaller than those in Expt. 1, particularly in the traps (Smith & Rennison, 1961); the data could not be analysed by $1\frac{1}{2}$ -hr. catching periods because of the frequency of zero values. However, the periodicity of the catches on oxen and in traps (fig. 2) was similar to that in Expt. 1 although the sexes differed less and the highest catches on oxen were from 0800 to 0930 hr. instead of 1700 to 1830 hr.

It is evident from comparison of figs. 1 and 2 that catches of males between 0800 and 0930 hr. on an ox were the same in both experiments and that the equivalent trap catches differed by only a small amount, but that catches later in the day were lower in Expt. 2, particularly in the last catching period.

The dissimilarity in both experiments between the numbers of flies taken on the fly-round and those caught on oxen and in traps has been discussed (Smith & Rennison, 1961). The fly-round catches showed no significant change from experiment to experiment but the catches on the oxen and in the traps did; this reflects the fact that whereas catches of males between 0800–0930 hr. by either method were much the same in both experiments, catches later in the day were lower in the second experiment (figs. 1 & 2).

The fly-round data in fig. 2 are means of the transformed values of the numbers of flies caught on the experimental section of the fly-round during each $1\frac{1}{2}$ -hr. catching period (Smith & Rennison, 1961). In the case of females, the curve closely follows that on oxen, but in the case of males, fly-round catches differ from those on oxen, the numbers decreasing during the day from the second period onward.

The relationship between the methods of catching.

Moving averages of the temperatures and saturation deficits recorded every $1\frac{1}{2}$ hr. from 0800–1830 hr. in each experiment are depicted in figs. 1 and 2. In Expt. 1, the curves of the numbers of females captured in brown and black traps closely followed the temperature ($P < 0.01$) and saturation deficit ($P < 0.01$), as did the more skewed curves for males. The catches of females on an ox were negatively correlated with saturation deficit ($P < 0.05$) but not with temperature, and male catches were correlated with neither. Female catches in both colours of traps were negatively correlated with the equivalent catches on the cattle ($P < 0.02$) but male catches for the two attractants were unrelated. We interpret these data to mean that when the temperature and saturation deficit were greatest, tsetse availability and activity were least in the population with which we were working. However, under these conditions the trap catches, particularly of females, were largest, a finding that does not concur with the generally accepted hypothesis that tsetse females are inactive between feeds and that this sedentary existence enables them to outlive the male population and outnumber it by some two to one (Jackson, 1937). It could be inferred, therefore, that females of *G. pallidipes* were attracted to traps not because these were regarded as a source of food but because some flies were compelled to seek resting places perhaps because of dryness, light and heat; only those insects that had to feed or die continued to come to the cattle. This view is supported, perhaps, by the finding of Jack (1941) that the fat content of females of *G. pallidipes* caught in a trap was higher than that of females caught on a bait ass.

The curve for females on cattle (fig. 1) suggests that they tended to be active throughout the day with only a small drop in numbers shortly after noon. We tentatively conclude, therefore, from the large numbers of females taken in the traps about this time (when, as shown by Bursell (1957) in the laboratory, spontaneous activity induced by dry conditions would be at a maximum) that they were being driven from their original resting places rather than that they were compelled to desist from hunting. For two reasons, however, it cannot be asserted that males were affected in the same way: first, it has been accepted that non-hungry males are readily attracted to moving objects in the expectation of finding mates (Vanderplank, 1948); secondly, Jack (1941) showed that the fat reserve in males taken in traps was lower than in those taken on a bait animal. From the data in fig. 1 it would seem that males, however 'hungry', were also seeking shade during the hotter and drier time of day, unless the catches at this

time represent that section of the male population which, being in a state of inanition (E. Bursell, The behaviour of tsetse flies (*G. swynnertoni*) in relation to problems of sampling *), are drawn to any object not forming a consistent part of the general habitat.

In Expt. 2 (fig. 2) the temperature and saturation deficit showed trends similar to those in Expt. 1, although values (except of early morning temperatures) were always higher. The mean catches of both sexes of *G. pallidipes* on oxen showed highly significant negative correlations with temperatures ($P < 0.001$) and saturation deficits ($P < 0.01$), but the trap catches, though positively associated with temperature and saturation deficit, were not correlated significantly with the former and only weakly so with the latter ($P < 0.05$). At the higher levels of saturation deficit, therefore, the more extreme temperatures apparently caused a decline in activity on oxen without a corresponding increase in trap catches. Perhaps all forms of activity were depressed at such levels of temperature and dryness, or perhaps a smaller proportion of the population existed in a state of non-appetitive activity. The slight peak of males in the trap catches during the third and fourth periods in Expt. 2 compared with the peak in the fifth and sixth periods of Expt. 1 suggests that the traps attracted some males as the appetitive response was waning.

In any event, it is obvious that *G. pallidipes* was not drawn to oxen and traps for similar reasons and the possibility that the traps took flies that were inactive (in the sense that they were unattracted to bait-animals) does not concur with the belief of others (Swynnerton, 1933, 1936; Harris, 1932; Morris & Morris, 1949) that the traps they had constructed caught flies because they resembled hosts, at least in outline. Without further investigation the term "animal trap", used by Morris & Morris (1949) to describe their trap, and accepted by Buxton (1955), should not be employed. Other species of *Glossina* may be attracted to these structures as hosts whereas *G. pallidipes* finds in them attributes of resting sites, but it seems unlikely.

The similarity, mentioned above, between the curve of the fly-round catches of females and the catches on the oxen provides further evidence that females are caught on fly-rounds primarily when hungry; the males, on the other hand, were probably not attracted to the patrol as a source of food. Physiological evidence on this point has been obtained by Bursell (quoted above), working with *G. swynnertoni* Aust.; he found that flies of either sex that were caught while probing on a bait-animal had low fat reserves, whereas flies attracted to a catching party consisted mainly of 'non-hungry' males and a few 'hungry' females.

These comparisons of methods of sampling tsetse populations were initiated to investigate the likelihood that Morris traps would offer a better measure than fly-round data of the density of *G. pallidipes*, with a view to their use in estimation of trypanosome challenge to cattle. It was concluded in the light of their dissimilarities that neither method was particularly appropriate. The pattern of catching throughout the day shown by these two methods and by catches on oxen strengthens the earlier conclusion. Indeed, the accumulated evidence emphasises that each method may be collecting a specific sub-sample which can comprise a variable proportion of the population at different seasons and at different times of the day, but firm assertions about the behaviour of tsetse in the field cannot be made in the absence of experimental evidence that samples collected simultaneously or consecutively in restricted areas are independent. The methods we employed do not preclude the possibility of non-random sampling, but they can be utilised to produce information on the problem of dependence between attractants, in addition to their other functions. A communication on this point will be made subsequently.

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Summary.

A series of catches of *G. pallidipes* Aust. was made in 1½-hr. periods between 0800 and 1830 hr. each day during two experiments carried out in the early wet season (1957) and the late dry season (1958), at Lugala, Uganda, using tethered, small, shorthorned East African Zebu oxen, Morris traps and the standard fly-round technique.

Flies were attracted to the oxen in greater numbers in the morning and evening than at midday, the evening increase being marked in the wet season. The daily catches of both sexes on oxen, though starting at much the same level in both seasons, fell to lower levels at the hotter times of day during the dry season and rose only slightly in the evening. Traps, on the other hand, in both seasons caught most females between 1230 and 1400 hr. and least in the mornings. Male flies were trapped in greatest numbers between 1400 and 1530 hr. in the wet season, but only in comparatively small numbers at any time in the dry season, though there was a suggestion of maximum availability between 1100 and 1230 hr. during the latter. During the dry season, catches on the fly-round and on oxen showed a similar periodicity in the case of females, but not in that of males, fly-round catches of which declined from a peak at 0930–1100 hr.

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SOME FACTORS AFFECTING NUMBERS OF *EMPOASCA LYBICA*
(DE BERG.) (HOMOPTERA: CICADELLIDAE) INFESTING
COTTON IN THE SUDAN GEZIRA.

E. H. J.

By R. J. V. JOYCE*

Desert Locust Survey, Nairobi, Kenya.

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Introduction.

The Sudan Gezira is a roughly triangular area of some five million acres, bounded on two sides by the Blue and the White Niles, south of their confluence at Khartoum, and on the third by the railway from Sennar to Kosti. Apart from a few rocky outcrops, especially in the southern extremities, it is a flat plain, the slopes for the most part being so gentle as to be scarcely visible. The soil is a heavy, alkaline, cracking clay and stores little rainwater available for plant growth during the prolonged dry season. The mean annual rainfall at Sennar, the southern extremity, is 450 mm., with a mean annual variation of 16 per cent., the corresponding values at Wad Medani being 400 mm. and 22 per cent., and at Khartoum 160 mm. and 34 per cent., respectively. Between 60 and 70 per cent. of the season's rain may be expected in July and August, when relative humidity is high, the mean at 0800 hr. at Khartoum, Wad Medani and Sennar being 57, 67 and 76 per cent., respectively, for July and 68, 75 and 70 per cent. for August. With the cessation of the rains and onset of the dry northerly winds, humidities fall abruptly during October, and are lowest in April, when the means at 0800 hr. are 18, 20 and 34 per cent. for Khartoum, Wad Medani and Sennar, respectively.

* Formerly of Agricultural Research Division, Ministry of Agriculture, Republic of the Sudan.

The annual mean temperatures are 29.6, 28.5 and 28.7°C. for Khartoum, Wad Medani and Sennar, respectively. The hottest month, when 44°C. may be recorded, is April in Wad Medani and Sennar, and May in Khartoum. The coldest month at all these stations is January, with a mean minimum temperature around 14°C., but temperatures of 5–6°C. occur frequently.

During the rains, herbs and coarse grasses flourish, but during the dry season the land is bare apart from trees, of which *Balanites aegyptiaca* and various species of *Acacia* are most numerous, especially in the south, while *Zizyphus* spp., *Capparis decidua* and *Cadaba farinosa* may be common in scattered patches and along river banks. Apart from such trees, which maintain their vegetative growth throughout the year and are especially vigorous at the onset of the rains, the Gezira during the dry season is almost devoid of green vegetation, which can flourish only along the banks of the Blue and the White Niles and the tributaries of the former, the Rahad and the Dinder, or where irrigation water, from the Sennar dam or from pumping stations, is supplied to cotton (until April) and to private gardens (throughout the year).

The cotton jassid,* *Empoasca lybica* (de Berg.), is a major pest of cotton in the Sudan Gezira, where over 300,000 feddans† of *Gossypium barbadense* are grown on Government and private estates and all is liable to more or less severe damage. The largest and most important of these estates is cropped by the Sudan Gezira Board (S.G.B.), with nearly 239,000 feddans annually under cotton.

In the Gezira, cotton is normally sown between the 12th and 30th August, and populations of 30,000–45,000 plants, in 10,000–11,000 plant-holes, are attained per feddan. Young cotton is colonised within a few days of its emergence by adults of *E. lybica*. Breeding continues in the crop until cotton plants are removed in May, populations increasing broadly exponentially until late November or mid-December and then declining to a low level, which is maintained, with a slight resurgence in February, until the end of the cotton season. Thus, the period during which *E. lybica* is of economic importance is approximately 1st September to 15th December, when its population may have reached from one million to ten million per feddan.

The early records of *E. lybica* in the Gezira were summarised by Snow and Taylor (1952), who suggested that it has always been numerous on cotton, at any rate since 1929, when it first received attention. Its possible economic importance was demonstrated by cage experiments in 1935 and by field experiments in 1942–1945 (Cowland, 1947). Subsequent large-scale trials (Cowland & Edwards, 1949) led to the adoption of large-scale control by spraying with DDT, the commercial success of which was demonstrated by Snow & Taylor (*op. cit.*).

The life-history of *E. lybica* in the Sudan was first studied by Michelmore (1930), who found the egg and nymphal stages to be of short duration and followed by a long adult life. Cowland (1947) gives the shortest duration of the egg stage as six days and the average as 8–10 days, and the nymphal stage as 8–12 days. This has been confirmed by the writer, although observations were not continued into the cold-weather months. Eggs are inserted chiefly in the main veins of the leaves of cotton plants and sometimes in the stem. In cages, batches of 1–7 nymphs hatched at intervals of 3–6 days and adults lived for up to 40 days. These observations were made between June and October, when monthly mean temperatures ranged from 39.5° in June to 33.6°C. in August. There were no indications that the range of temperatures experienced greatly influenced the duration of egg or nymphal stages, or caused high egg mortality as

* The family to which *Empoasca* belongs is now correctly known as CICADELLIDAE, but the name jassid has for so long been applied to the species infesting cotton that it is, for convenience, retained here.

† One feddan=1.038 acres=0.42 hectares. The area under cotton has increased substantially since this paper was drafted, as a result of the development of new irrigation projects.—Ed.

reported by Klein (1948) for temperatures above 27°C. Similarly, no nymphal deaths were observed on cotton or egg-plants (*Solanum melongena*) grown in cages at temperatures considerably in excess of 27°C. In adult collections, females outnumbered males (2:1). The pre-oviposition period of the adult during the months when observations were made was 2-4 days.

From these data it is possible to compute the theoretical rate of increase that would occur if all progeny lived their full span of life (*cf.* Thompson, 1931). Fig. 1A is a graphical representation of the number of nymphal progeny from a

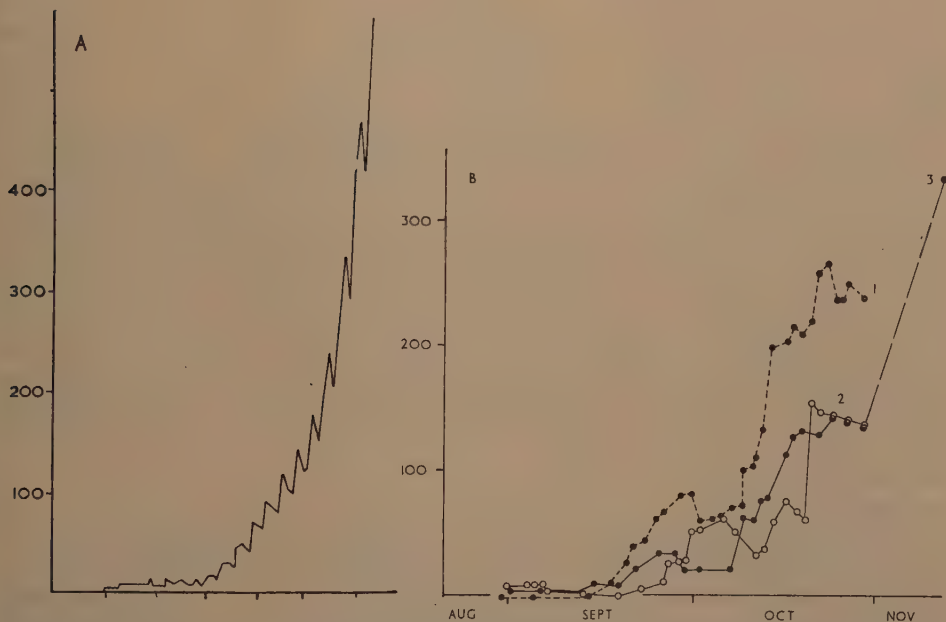


Fig. 1.—A, theoretical rate of increase of populations of *E. lybica* (the origin is at day d and each interval represents 10 days); B, recorded rate of increase of populations of *E. lybica* on cotton (2 and 3) and on *Solanum melongena* (1), derived from single pairs of caged males and females.

pair of newly emerged adults, assuming that the first batch of eggs was laid on day d , were nymphs from $d+9$ to $d+18$, and became adults (pre-oviposition) on $d+19$. It is assumed that the sex ratio is 2 females:1 male, and that each female lays 2 female plus 1 male egg on $d+20$, $d+23$, $d+26$ $d+77$, when both males and females die, after 60 eggs have been laid. In fig. 1B is plotted the recorded number of nymphs from pairs of males and females introduced to either cotton or egg-plant (*Solanum melongena*) in cages, where breeding was allowed to continue for 60 or 75 days. Both computed and actual nymph populations increase by a series of jumps interrupted by periods of decline coinciding with transformation of nymphs to adults. Thus it may be calculated that, in the period of approximately 100 days (early September to mid-December) available for breeding on the crop, a single pair of *E. lybica* colonising cotton could give rise to a population of 47,050, of which 26,838 would be nymphs and 20,212 adults. If the rate of egg-laying were increased so that the female produced the same number of eggs (60) in 20 days, instead of 60, a pair of *E. lybica* would give rise, during 100 days, to 1,246,000 progeny, of which 917,000 would be

nymphs. This number would not be reached by the pair breeding at the slower rate until 20 days later.

It is clear that the populations of *E. lybica* recorded on cotton could be accounted for adequately by the reproduction of a small number of immigrants to young cotton, and the elucidation of the factors that influence the attainment of the full reproductive potential is thus of considerable economic importance.

In the estate cropped by the Sudan Gezira Board, Hanna (1950) noted that in the northern area (lat. $14^{\circ} 55'$, long. $33^{\circ} 15'$) jassids were always numerous and caused heavy damage; in the central areas (lat. $14^{\circ} 20'$, long. $33^{\circ} 25'$) they were always present but only occasionally reached pest proportions and in the southern area they were present in small numbers and only rarely caused damage. Observations indicated that heavy rainstorms reduced populations of jassid nymphs and experiment proved that this effect was produced by mud splash. Subsequent observations confirmed these conclusions and a striking instance of the harmful effect of heavy rain in September on jassid populations was reported by Goodman & Toms (1956). Hanna (1950), moreover, showed an impressive inverse relationship between peak jassid infestations recorded on experimental plots of cotton in northern and central Gezira and the amount of rain falling in storms of 10 mm. and above in these areas during July and August. This finding permits the inference to be drawn that the population on cotton is determined by the extent of initial colonisations, and accordingly it should be possible to prevent infestations of economic importance by reducing jassid populations during July and August, before the cotton is sown. Moreover, Cowland & Hanna (1950) concluded that irrigated gardens within the Gezira were the chief source of infestations of cotton and that control of the pest could be achieved before cotton was colonised and without spraying the crop. Investigations of this possibility are reported in the present paper.

Seasonal history of *Empoasca lybica*.

Host-plants.

Cowland (1947) listed 14 species of host-plants, in four families, on which *E. lybica* was recorded as having bred to maturity, and eight species, in three other families, on which adults were recorded but on which it was uncertain that the life-cycle could be completed. Cowland & Hanna (1950) added eight more host-plant species, in six additional families, on which *E. lybica* bred to maturity, and 12 more species on which adults could live. Attempts by the author to breed *E. lybica* on a number of common Gezira weeds resulted in a further extension of the list of host-plants.

The following list of host-plants recorded in the Sudan Gezira, although doubtless incomplete, is considered to include all those important in the local ecology of *E. lybica*; it comprises some 53 species from 20 families.

The survival of *E. lybica* under the excessively severe conditions of the dry season was studied by Cowland & Hanna (1950) and continued by the writer. Repeated search and experiment failed to establish the occurrence of diapause in any stage of development, a finding consistent with that of Klein (1948). Soil cracks, which provide dry-season retreats for many insects in the Sudan, including grasshoppers (Joyce, 1952a), were not inhabited by *E. lybica*. The areas of perennial irrigation such as gardens and river banks were found to harbour *E. lybica* throughout the dry season and are considered to be the permanent breeding grounds of the species. Accordingly, observations were made on the occurrence of *E. lybica* in these sites, especially in May–July, when the cotton plants have been pulled out and burnt.

During the seasons 1949 and 1950 a total of 25 Gezira gardens (13 in northern, 2 in central and 10 in southern Gezira) were inspected monthly during May–August. Each garden was 10 feddans in area and was divided into 16 equal

squares which were thoroughly searched for host plants. Infestations were classed as heavy, medium, light or absent, and the area occupied by each was measured. Heavy infestations occurred only on hosts, such as egg-plant, on which counts could be made, and it was therefore possible to record the total population of

Family	Breeding recorded	Adults only recorded
Malvaceae	<i>Gossypium</i> (all species) <i>Abutilon glaucum</i> <i>Abutilon</i> spp. <i>Sida</i> spp. <i>Hibiscus esculentus</i> <i>H. sabdariffa</i> <i>H. cannabinus</i> <i>Hibiscus</i> spp.	
Portulacaceae	—	<i>Portulaca quadrifida</i>
Cucurbitaceae	<i>Cucurbita pepo</i> <i>Cucumis melo</i>	
Tiliaceae	—	<i>Corchorus olitorius</i> <i>Corchorus</i> spp. <i>Gisekia pharnaceoides</i>
Molluginaceae	—	
Lythraceae	<i>Lawsonia alba</i>	
Myrtaceae	<i>Psidium guajava</i>	
Euphorbiaceae	<i>Ricinus communis</i>	<i>Euphorbia aegyptiaca</i> <i>E. acalyphoides</i> <i>Phyllanthus niruri</i> <i>Acalypha indica</i> <i>Acacia arabica</i> <i>Crotalaria</i> spp. <i>Medicago sativa</i> <i>Clitoria ternatea</i> <i>Phaseolus</i> spp. <i>Vigna unguiculata</i> <i>Vigna</i> spp. <i>Dolichos lablab</i> <i>Cajanus cajan</i> <i>Tephrosia uniflora</i>
Mimosaceae	—	
Papilionaceae	<i>Vicia faba</i> <i>Rhynchosia memnonia</i>	
Simarubaceae	<i>Balanites aegyptiaca</i>	
Rhamnaceae	<i>Zizyphus spina-christi</i> <i>Z. mucronata</i>	
Ampelidaceae	<i>Cissus quadrangularis</i> <i>Cissus</i> spp.	
Compositae	<i>Helianthus annuus</i>	
Solanaceae	<i>Solanum dubium</i> <i>S. incanum</i> <i>S. melongena</i> <i>Capsicum frutescens</i> <i>Withania somnifera</i> <i>Datura</i> spp. <i>Lycopersicum esculentum</i>	
Convolvulaceae	—	<i>Ipomoea blepharosepala</i> <i>I. cordofana</i> <i>Sesamum orientale</i> <i>Ocimum basilicum</i>
Pedaliaceae	—	
Labiatae	—	
Cannaceae	<i>Canna indica</i>	<i>Sorghum vulgare</i> <i>Echinochloa colonum</i>
Gramineae	—	

nymphs on a sample of plants, the number of plants and the area occupied. Heavy populations averaged about 1,000 nymphs per square yard. Populations judged as medium were dealt with in a similar manner and occurred usually on convenient plants, although considerable difficulties were encountered with fruit

trees, such as guava. Numbers classed as medium were generally about one-tenth of those classed as heavy, that is, 100 per square yard. Populations judged as light were especially difficult to estimate. Attempts were made to count all individuals, to kill them by spray, and to collect them by sweeping with nets. The last method always gave the highest estimate and was adopted. Populations classified as light supported on an average about 50 adults of *E. lybica* per 100 square yards.

Such figures are not suitable for statistical analysis, but those obtained in 1949 are summarised in Table I, and suggest the range of populations considered likely to occur. The estimated populations in gardens examined in 1950 were generally of the same order (Joyce, 1952b).

TABLE I.

Estimated populations of *E. lybica* in irrigated gardens, Sudan Gezira, 1949.

Locality	Population (thousands per feddan)					Rainfall (mm.)	
	May	June	July	Aug.	Sept.	Total	In storms of over 10 mm.
Northern Gezira							
Badr	tr.	Nil	1	Nil	Nil	139	124
Turabi	Nil	tr.	1	tr.	N.A.	91	80
Rukn	tr.	tr.	2	N.A.	N.A.	109	92
Wad Hussein ..	24	27	109	4	151	113	95
Central Gezira							
Soriba	106	4	2	2	1	147	109
Hamad el Nil ..	46	12	1	4	1	174	154
Southern Gezira							
Ghubshan	tr.	3	tr.	50	1	85	29
Wad Naaman ..	Nil	1	1	2	1	236	187
Bolein	tr.	tr.	2	2	tr.	181	164

tr.=infestation estimated at less than 500 per feddan.

N.A.=no record made.

Of over 51,000 specimens of *E. lybica* recorded by examination of 53 known species of host-plants in 18 gardens during four months during 1950, over 44,000 were recorded on *S. melongena*, *S. dubium* and *Abutilon glaucum*, which accounted for 39, 15 and 31 per cent., respectively. The percentage of the total on *Hibiscus* spp. was low because, for phytosanitary reasons, the growth of these species is prohibited. The mean infestations per plant were 24 for *S. melongena*, 4.5 for *S. dubium*, 2.8 for *Hibiscus* spp. and 2.3 for *A. glaucum*. Further details are given by Joyce (1952b). In spite of the very small numbers and sizes of these favoured host plants, their presence appeared to be virtually the sole factor determining the size of populations in gardens, other host plants making unimportant numerical contributions. The most important of the three is *S. melongena* which is grown only in small plots of few plants. *S. dubium* and *A. glaucum* were seldom numerous, and were most frequently recorded on parts of the gardens that were seldom irrigated. *S. dubium* was preponderant in the drier, northern areas and *A. glaucum* in the wetter, southern ones. Populations of *E. lybica* recorded in gardens were thus extremely low, ranging from nil to some 150,000 per feddan and being determined apparently by local custom concerning host-plants. There was no suggestion of any correlation between estimated population and rain storms.

Surveys of favoured host-plants were also attempted on river banks. In the immediate vicinity of Wad Medani during August 1952, many small plots of *S. melongena*, probably 2-5 months old, were recorded, in 20 gardens, and the mean infestation exceeded 150 nymphs per plant.

On the White Nile there are few perennially irrigated gardens, if any. However, in this area *E. lybica* was found throughout the dry season on tree hosts growing near the river, of which *Zizyphus* spp. were most numerous. Moreover, throughout the Gezira, in northern Fung (that is, south of the Sennar-Kosti railway) and on the east bank of the Blue Nile, particularly along its tributaries, the Dinder and the Rahad, and in the numerous, seasonally flooded forests of the horse-shoe loops of all these rivers, *Balanites aegyptiaca* is common or dominant. Populations of *E. lybica* were frequently recorded on this host in the dry season and increased when fresh vegetative growth appeared at the onset of the rains.

The extensive occurrence of host-plants in riverain vegetable gardens and the wide distribution of tree hosts make it possible to assert with confidence that extensive sources of infestation of *E. lybica* exist other than those provided by the irrigated gardens of the Sudan Gezira Board, though making it impracticable to delimit the range of such additional sources or to arrive at a numerical estimate of their importance.

Flight activity.

Proof of colonisation of a particular area by insect movement from a distant source of infestation can be obtained only by marking and recapturing. Nevertheless, long-distance movement can be inferred with confidence under certain conditions, as in the case of the migration of the leaf-hopper, *Circulifer tenellus* (Baker), in California, over a distance of some 400 miles (Dorst & Davis, 1937; Fulton & Romney, 1940). The occurrence of wind dispersal (as opposed to the distance over which it occurs) can be demonstrated by trapping (Freeman, 1945; Johnson, 1950, 1951, 1952, 1956; Williams, 1952). Johnson (1956) drew attention to the importance of the duration of unstable air conditions in promoting or limiting dispersal, and instability is a characteristic feature of air over the Gezira, especially during the rains (Ireland, 1948).

Long-distance dispersal of *E. lybica* in the Sudan could be inferred from many observations on the occurrence of infestations, such as those on rain-grown *S. dubium* during September in unirrigated parts of the Gezira, 20 miles from the nearest cotton or perennial vegetation, or in October in the Butana, the country lying between the Blue Nile and River Atbara, which supports, over much of its expanse, tall annual grasses, which are burnt off during the dry season, leaving an area completely devoid of vegetation (Joyce, 1952*a*). The latter infestations were perhaps 100 miles from the nearest perennial vegetation known to support host-plants of *E. lybica*. Further circumstantial evidence is provided by infestations in October on isolated cotton plants grown ornamentally in a rain-fed garden on the southern edge of the Butana, and on *Cissus quadrangularis* and *S. dubium* in February on the Red Sea littoral, 50 miles from the nearest cotton and an unknown distance from perennial host-plants, which probably occurred in khors in the Red Sea hills.

Flight activity was accordingly investigated by means of sticky traps (Broadbent & others, 1948) and a light-trap (Williams, 1948), although Cowland & Hanna (1950) had failed to capture any adults of *E. lybica* with the former, when mounted at a height of 15 feet, during the rains in 1948.

During 1949, eighteen sticky traps were erected on different fallow phases on the Gezira Research Farm (G.R.F.) and one on top of an 80-ft. water-tower in Wad Medani, about half a mile away from the nearest known source of infestation. The traps were 4-in. diameter cylinders mounted on steel poles at heights of 4-6 feet, and each was covered by a celluloid sheet 1.5 sq. ft. in area. On alternate

days these sheets were removed, placed in boxes and brought to the laboratory for examination. Observations were made from the end of June until the 20th October, by means of three such traps mounted in each of the following 5-feddan plots: fallow after cotton, fallow after sorghum, sorghum, cotton, continuous fallow and lubia (*Dolichos lablab*). There were no significant differences between the catches from any plot, so the results were lumped together.

In 1950, twenty traps were used, mounted at a height of 2½ ft. and painted green. Ten were placed on each of two 5-feddan fallow plots and observed from June to the 14th December.

The catches are summarised in Table II.

TABLE II.

Monthly catches on sticky traps, G.R.F.

	1950				1949	
Month	Total jassids	<i>E. lybica</i>			Total jassids	Total <i>E. lybica</i>
		Male	Female	Total		
June	244	6	42	48	No records	
July	218	24	54	78	162	38
August	182	6	33	39	113	6
September	434	3	7	10	47	5
October	1111	6	21	27	25	0
November	268	0	4	4	No records	
December (first half)	44	0	10	10	No records	
Total	2501	45	171	216	347	49

The numbers captured between June and November were much less in 1949 than in 1950. In 1949 nearly 80 per cent. of the total were captured before the end of September. In 1950 substantial flights of jassids occurred in June and July, but peak numbers were captured during October. Most of the catch consisted of an unidentified species which was recorded on *Brachiaria*, a type of grass common in 1950, when rains were heavy, and infrequent in 1949, when rains were poor. Thus, the peak catches in October 1950 may have resulted from movement of individuals, bred in the rains, as their host-plants dried up, and the absence of an October peak in 1949 may have resulted from reduced breeding on the limited numbers of host-plants which followed the poor rains.

The proportion of the total captures of *E. lybica* that was effected before 1st September (before cotton, sowing of which began on 15th August, could have been a factor in the ecology of the insect) was over 85 per cent. in 1949 and over 76 per cent. in 1950. The low catches from September onwards, when total populations in the area were increasing enormously as a result of breeding on cotton, suggest that once cotton is colonised, flight activity of *E. lybica* is reduced. This suggestion finds support in the records of 1949 when, from September onwards, no more examples of *E. lybica* were trapped on 5-feddan cotton plots, having 33,000 host-plants per feddan, than on fallows, which from September onwards supported almost no host-plants.

Catches of *E. lybica* in light-traps in 1949, when rains were poor, yielded 4,729 adults between July and October, inclusive, of which nearly 54 per cent. were collected before 1st September. In 1950, catches were frequently destroyed through flooding of the containers in heavy storms.

On the sticky trap mounted on the 80-ft. tower at Wad Medani in 1949, nine

jassids were caught, including five examples of *E. lybica*. Four of these were collected between the 12th and 30th July, and one on the 9th September.

These trap records confirm the occurrence of wind dispersal of *E. lybica*, but give no indication as to distance covered. The capture of specimens at a height of 80 feet on a single small trap suggests the occurrence of large aerial populations and the possibility of dispersal over great distances.

Occurrence of *E. lybica* on Gezira fallows.

The Gezira is cropped on an 8-course rotation: cotton, fallow, dura (*Sorghum vulgare*), lubia (*Dolichos lablab*), fallow, cotton, fallow, fallow. Thus, half the area rests as uncultivated fallow each season, or more than half if, as is frequently the case, a further fallow is substituted for *Dolichos*. The rotating unit is termed a "Number", which is an area of 90 feddans, divided into nine 10-feddan fields.

For most of the year the fallows, which are heavily grazed, especially in the north, are bare, the deeply cracked black soil being completely devoid of green vegetation and the surface temperature frequently reaching 65°C. The rains may promote weed growth at any time between June and August; the seasonal variability is very great, especially in the north, where sometimes rains may be completely ineffective.

Surveys of the incidence of *E. lybica* on host-plants in fallows were made during July, August and September, in 1949, 1950 and 1951.

In 1949, the survey was made on 2-4 ten-feddan fallow fields from each of five of the phases of the Gezira rotation, both in areas near the irrigated gardens examined during the same season and in areas as far removed as possible from such sources of infestation. These surveys were conducted near to the G.R.F. and in seven of the 40 administrative Blocks into which the S.G.B. estate was then divided. Thus, a total of 360 ten-feddan fields scattered throughout the Gezira was sampled. Observers walked diagonally across each field, dropping a square measuring 0.25 sq. ft. at every eighth pace. Each weed within the square was plucked individually and examined for jassids; any found were recorded and preserved in alcohol for subsequent identification in the laboratory. The results are summarised in Table III.

TABLE III.

Numbers of nymphs of *E. lybica* (per 50 sq. yd.) on weeds in fallows before germination of cotton, Sudan Gezira, 1949.

Gezira locality	Fields near gardens					Fields away from gardens				
	FF	CF	DF	C	D	FF	CF	DF	C	D
North	0	3	0	2	11	35	4	0	11	19
Centre	0	0	0	0	36	0	0	0	0	0
South	6	1	31	3	5	8	0	0	21	5
Total	6	4	31	5	52	43	4	0	32	24

The letters represent phases of the rotation: C (Cotton), F (Fallow), D (Sorghum), L (*Dolichos*). The letters in heavy type indicate the treatment scheduled for the field during the season of observation; the others indicate the treatment during the previous season.

In spite of the wide range of recorded weed host-plants in the Gezira, almost all of the 201 examples of *E. lybica* collected in the survey were taken on *Solanum dubium* and *Rhynchosia memnonia*, mostly on the former. Both weeds were distributed very erratically and occurred typically in localised patches, such as the tops and sides of watering channels, so that the system of sampling by

random squares failed to provide an estimate of their incidence. It was noteworthy, however, that *E. lybica* was already widely distributed on fallows near to and far from known sources of infestation before cotton germinated, and there were indications that the sorghum phase, which was irrigated at the time of the survey, might be an important breeding ground of *E. lybica*. A special examination of four 10-feddan sorghum fields in northern Gezira revealed a total of 650 sq. yd. to be heavily infested with *S. dubium*, on which were found 681 examples of *E. lybica*.

In 1950, the survey was confined to recording the total number of plants of *S. dubium* and *R. memnonia* on ten Numbers of fallow following cotton, ten of fallow following fallow and ten cropped with sorghum. The population of nymphs of *E. lybica* was estimated by counting the numbers occurring on ten host-plants taken at random. This survey was carried out in two Blocks in northern Gezira, in one of which the Estate administration had made a special attempt to eradicate these weeds, one Block in north-central Gezira, and one Block in southern Gezira of which half was subject to intensive weed control in the fallow phase. The results obtained are given in Table IV.

TABLE IV.

Incidence of *Solanum dubium* and *Rhynchosia memnonia* on certain phases in the Gezira rotation, with recorded infestations of *E. lybica*, 10.viii-15.ix.50.

Locality and weed control	Estimated host-plants per feddan			Mean number nymphs per 100 host-plants			Area of Block in given phase (feddans)		
	FF	CF	FD	FF	CF	FD	FF	CF	FD
North Gezira									
A	0.3- 13.4	0- 468	0- 248	6.6- 604	0- 112	0- 320	860	1700	860
B	4.6- 90.2	3.0- 315.4	0- 83.5	0- 59	0- 177	0- 43			
North-Central Gezira									
B	41.4- 599.0	52.4- 522.4	No counts	12.0- 470	5.3- 37	No counts	1200	2400	1200
South Gezira									
A	No counts			No counts			300	600	300
B	0- 284.2	14.3- 4030	0- 269.8	0- 380	0- 597	0- 400	300	600	300

A. Intensive eradication of weed hosts in fallows.
See Table III for explanation of lettering.

B. No control of weed hosts in fallows.

The variation between plants in the numbers of nymphs of *E. lybica* was too great to warrant further analysis of the data. The results showed, however, that the host-plants were widely distributed but not uniformly abundant and cast doubts on the practicability of substantially reducing sources of infestation. Consideration of the area occupied by fallows in the Gezira rotation, and of the possibility of heavy infestation of *E. lybica* on even the small numbers of host-plants recorded, suggests that fallows may provide an even more important immediate source of infestation of cotton than the irrigated Gezira gardens.

Because of the inverse relationship that Hanna (1950) demonstrated between July-August rainstorms and peak infestation of *E. lybica* on cotton, and because the numbers of *E. lybica* in gardens appeared to be related to the availability of

preferred host-plants rather than to any climatic factor, it seemed desirable to examine, in relation to rainfall, the data on infestations on weed host-plants in cultivated areas. Accordingly, 100 ten-feddan fields from equal numbers of fallows following sorghum, fallows following fallows, fallows following cotton and fields cropped with sorghum, in each of three Gezira localities, were examined each month in June–October 1951. In each field, counts were made of the area occupied by three host-plants, *S. dubium*, *R. memnonia* and volunteer seedlings of cotton (which were very common that season), the mean number of each per square yard, and the number of nymphs of *E. lybica* recorded on 100 of each species of host. The results, together with the monthly rainfall, are given in Table V.

TABLE V.

Host-plants and nymphal populations of *E. lybica* recorded in 100 ten-feddan fields, and rainfall, in three Gezira localities, 1951.

Block	June	July	August	September	October
Total host-plants					
Wad Naaman (South)	0	0	5673	122725	108680
Tebub (Centre) ..	0	16349	7973	39404	132130
Istarahna (North) ..	0	25690	25125	234636	68506
Total nymphs					
Wad Naaman ..	0	0	7	5233	140405
Tebub ..	0	0	0	895	251
Istarahna ..	0	0	67	4533	632
Total rainfall (mm.) (means of 3–4 gauges per locality)					
Wad Naaman ..	42	52	254	56	12
Tebub ..	29	18	164	41	14
Istarahna ..	14	12	128	54	13

After transformation of the estimated population to $\log(n+1)$, an analysis of covariance of nymphal and host-plant populations suggested a tendency for the monthly increase in estimated nymphal population to be related to the monthly changes in host-plant populations, but the correlation ($r=+0.5985$) was not significant. The correlation of host-plant population with accumulated rainfall ($r=+0.5737$) was likewise not significant. This is not surprising, since many of the host-plants were on irrigated land under sorghum, and populations recorded on one occasion would disappear on the next as the cultivator weeded his crop. Moreover, owing to a combination of unusual factors, volunteer cotton seedlings were far more common than usual but were eradicated as soon as reported. Important causes of variance other than rain thus existed, which could not be measured. The estimated population of nymphs was significantly and positively correlated with accumulated rainfall from June to October ($r=0.8978$; $P=0.02$); this may reflect merely an increase of both with time, but it seems possible that the increased host-plant numbers might have had an advantage to *E. lybica* that outweighed any harmful effect of rainstorms.

Thus in two seasons (1949 and 1951) when the rainfall was below average, and in 1950, when it was above average, host-plants on Gezira fallows, though relatively few, were frequently, and often heavily, infested with nymphs of *E. lybica*. The Gezira fallows thus appeared at least as important as irrigated gardens as immediate sources of infestation of *E. lybica*.

TABLE VI.
Distribution of leaves and nymphs of *E. lybica* on cotton plants at different ages, 1949.

Zones (main-stem nodes)	No. of leaves on day					1st instar nymphs per zone on day				Total nymphs (all instars) per zone on day			
	40	51	63	76		40	51	63	76	40	51	63	76
A (1-5) ..	4.1	4.8	2.7	0.2		Nil Nil	0.14 0.03	0.02 0.01	0.03 0.15	0.03 0.01	0.22 0.05	0.11 0.04	0.03 0.15
B (6-10) ..	5.0	5.0	5.6	3.6		0.25 0.05	0.90 0.18	0.09 0.02	2.97 0.81	0.28 0.05	1.44 0.29	0.90 0.18	4.33 1.20
C (11-15) ..	1.0	2.6	6.1	6.2		0.03 0.03	0.56 0.19	0.72 0.12	6.42 1.04	0.03 0.03	0.64 0.25	2.68 0.44	10.06 1.62
D (16-20) ..	—	0.2	5.6	7.4		—	0.02 0.10	1.34 0.24	10.80 1.46	—	0.02 0.10	3.34 0.59	16.20 2.20
E (21-25) ..	—	—	3.6	5.7		—	—	0.72 0.20	11.44 2.02	—	—	2.08 0.59	15.31 2.70
F (26-30) ..	—	—	1.2	3.5		—	—	0.22 0.19	6.55 1.89	—	—	0.60 0.50	9.00 2.59
G (31-35) ..	—	—	0.9	0.6		—	—	—	1.03 1.72	—	—	0.03 0.03	1.32 2.20
Total ..	10.1	12.6	25.7	27.2		0.28	1.62	3.11	39.24	0.34	2.32	9.74	56.25

Figures in heavy type show mean number of nymphs per leaf, other figures represent total leaves or nymphs per plant.
Sowing date : 15th August 1949.

Occurrence of *E. lybica* on cotton.*Population distribution on the cotton plant.*

Cowland (1947) estimated jassid infestation by collecting adults in sweep nets and then counting them. Later he abandoned this method in favour of counts of

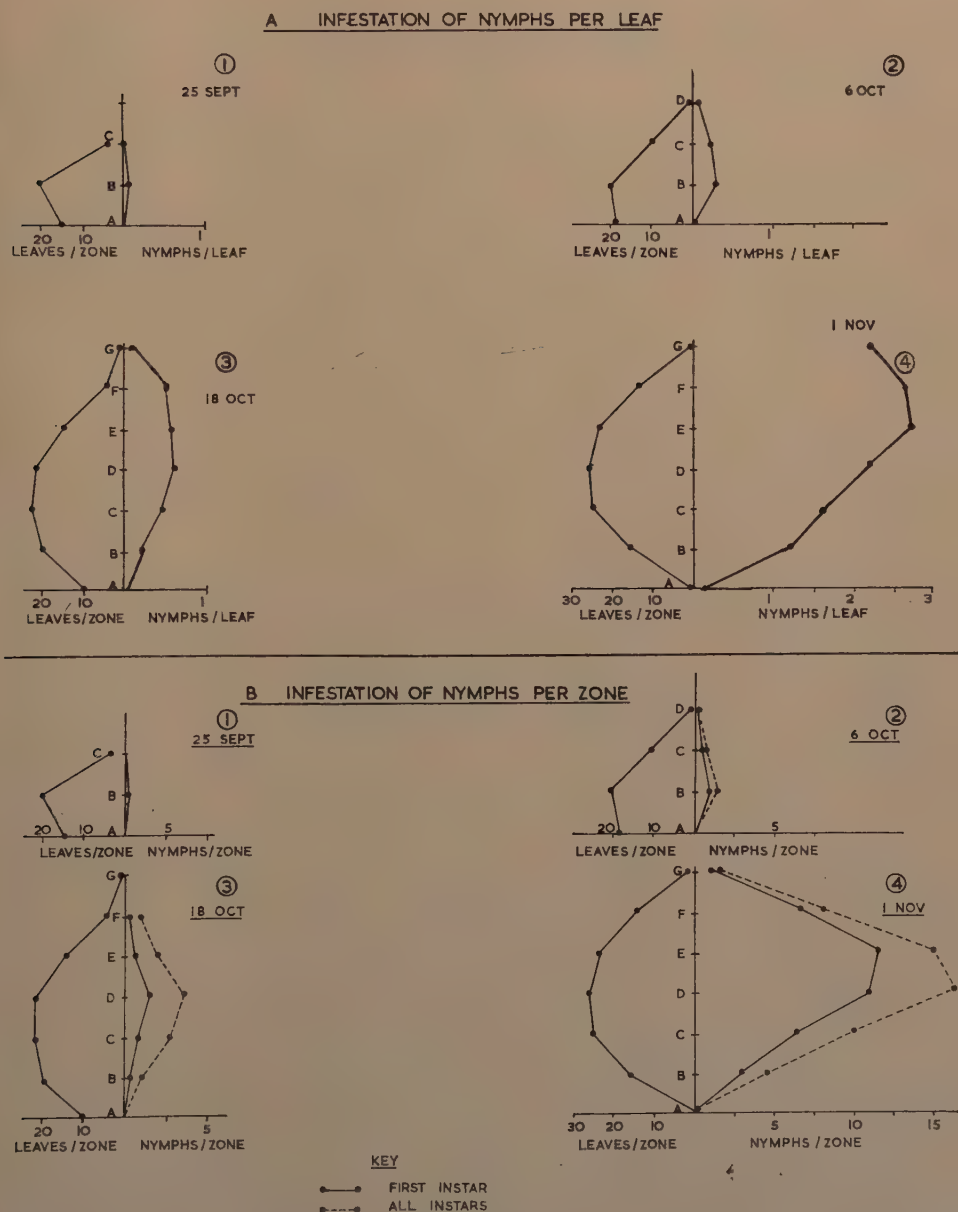


Fig. 2.—The distribution of nymphs of *E. lybica* on cotton plants on four different dates in 1949. Each of the zones, A to G, represents five main-stem nodes. Sowing date: 15th August 1949.

nymphs, which are less mobile, a practice continued by the writer. Since the adult female of *E. lybica* lays eggs in the leaf tissue in batches and is apparently discriminating in its choice of leaves, an irregular distribution of population on leaves was expected, especially amongst first-instar nymphs. Observations were made in 1949 on the distribution on cotton plants of nymphs classified into three age-groups (1st instar, 2nd and 3rd instars, and 4th and 5th instars). The numbers of nymphs of each age-group and the position on the plant of each leaf on which they occurred were recorded on four occasions, when the plants were 40, 51, 63 and 76 days old, using a three-factor experiment investigating two varieties, two levels of nitrogenous fertiliser and two spacings. Table VI shows the distribution of nymphs on the plant by zones, each zone representing five main-stem nodes, the oldest node being number 1 (see also fig. 2).

The nymphs were fairly evenly distributed amongst all zones except the oldest and the very youngest, but with a tendency for the highest populations to be on leaves 15–20 days old. A notable feature was that all zones participated in the big population increase that occurred between days 63 and 76.

The association of number of first-instar nymphs with number of leaves was investigated by an analysis of co-variance, in which the components due to age of, and zone on, the plant were eliminated. The residual co-variance had a correlation coefficient of 0.9052 ($P < 0.01$), indicating that over 80 per cent. of the variance in frequency distribution of numbers of first-instar nymphs could be positively accounted for by variance in the leaf-number distribution.

A similar analysis was made of the co-variance of the total number of nymphs (of all instars) and of leaves per plant, after eliminating components due to variety, fertiliser and spacing. The correlation coefficient of the residual co-variance was 0.8676 ($P < 0.01$), so that over 75 per cent. of the variance in distribution of all nymphs at all times was accounted for by the variance in leaf distribution.

The frequency distribution of number of nymphs per leaf at each date is summarised in Table VII. This distribution was significantly different from a Poisson series but approximated to a logarithmic series. This means that nymphs were not distributed over the plant at random, but were grouped. This grouping has been shown by the above analyses to be largely determined by the distribution of leaves. Thus, jassids, unlike cotton aphid (*Aphis gossypii* Glov.) and whitefly (*Bemisia tabaci* (Gennadius)), are most numerous in the leafiest zones of the plant and a random sample of leaves, over the area of infestation, seems to be a more appropriate method of sampling jassid infestations than that of random samples consisting of whole plants.

Population distribution in cotton fields and its relation to sampling methods.

In the Gezira, fields (termed Numbers) are typically 90 feddans in size, measuring 1350m. by 280m. and bounded on one long side by a water channel (Abu 20). Each is divided by a water channel (Abu 6) into nine 10-feddan tenancies (lettered A to I), each measuring 150m. x 280m. Cotton is grown on ridges 80 cm. apart and a tenancy should contain 185 ridges each 280m. long. Running across the ridges are minor water channels and holding banks which divide the tenancy into 14 or 16 (or, on the Gezira Research Farm, 20) equal areas known as angaias, each measuring 150m. x 20m. or 17.5m. (or, on the Research Farm, 14m.), an arrangement which facilitates irrigation. Cotton is sown on the ridge in holes 50 cm. apart, and thinned to a nominal three plants per hole. Thus, the plant population of 30,000–45,000 plants per feddan is contained in about 10,500 plant holes per feddan.

A series of counts was made on the Gezira Research Farm in which, from each of 10 to 136 plant holes selected at random in each of the 20 angaias of a 5-feddan plot, the total number of jassid nymphs on five randomly selected leaves (omitting

the very youngest and oldest leaves) was recorded. The results are tabulated in Table VIII. The mean number of nymphs per leaf as estimated from the 13,600-leaf sample (136 plant holes per angaia) was 0.224 (± 0.001) and from the 1,000-leaf sample (10 plant holes per angaia) was 0.226 (± 0.001). These results encouraged the view that an estimate of infestation could be obtained from a sample sufficiently small in size to be practicable. The method did not, however, enable the variance due to site (angaia) to be distinguished from the variance due to sampling. Accordingly, another series of counts was undertaken on com-

TABLE VIII.

Distribution of jassid nymphs across a Gezira Research Farm plot
(expressed as number of nymphs per 100 leaves).

Angaia	Size of sample per angaia	
	5 leaves from each of 136 plant holes	5 leaves from each of 10 plant holes
1	26.0	24.0
2	18.5	14.0
3	18.5	20.0
4	24.7	26.0
5	18.5	20.0
6	23.4	38.0
7	22.1	26.0
8	22.6	24.0
9	17.0	14.0
10	15.0	14.0
11	25.7	28.0
12	27.0	24.0
13	16.3	18.0
14	19.3	16.0
15	21.9	12.0
16	30.3	22.0
17	29.3	32.0
18	21.9	22.0
19	23.8	28.0
20	25.3	30.0
Mean	22.35	22.6
S.E.	± 0.100	± 0.147
Possible error for 95% confidence ..	9%	13%

mercially grown cotton in which, in each of the 14 angaias of two tenancies (B and F) of a 90-feddan field, a count was made of the total number of nymphs on five randomly selected leaves per plant station (there being at this time a mean of 68 leaves per plant station) from the central plant station, from 5 plant stations 2m., 10 plant stations 4m., and 20 plant stations 6m. from the central plant and, finally, from 100 plant stations selected at random in the angaia, each count being recorded separately. It was possible, therefore, to estimate for each method the variability per plant which could be ascribed to its site in the tenancy (angaia variance) or to sampling error (see Table IX). This analysis was made by P. Roberts, formerly Research Division Statistician.

In the case of tenancy B, the angaia variances, the sampling variances and the means for the different methods varied significantly amongst themselves. As the methods were intended to estimate the same things some of them must have been biased. The differences may have been due to bias in the selection of plants, or more likely because of real changes in jassid population, the counts

having been made over a period of eight days. The largest sample, which took the longest time, gave the highest mean. It is noteworthy that the two variances and the means tended to change together.

In the case of tenancy F, where counts were made a week earlier, the means

TABLE IX.

Jassid infestation in a standard Gezira tenancy (expressed as number of nymphs per 100 leaves, derived from 5 leaves per plant on n plants per angaia).

Angaia	Number of plants sampled per angaia (n)											
	Tenancy B						Tenancy F					
	1	5	10	20	100	136	1	5	10	20	100	136
1	100	152	153	161	246	222	40	36	40	63	83	75
2	180	160	245	165	224	211	20	48	42	41	72	64
3	20	100	125	142	184	169	60	68	40	54	65	62
4	200	192	133	111	184	170	60	96	54	32	47	47
5	120	116	173	110	178	164	100	68	44	50	69	65
6	180	112	157	124	181	169	40	52	58	40	64	60
7	140	128	140	140	171	162	60	56	78	77	92	87
8	140	108	125	99	144	135	40	32	64	41	106	90
9	100	68	95	96	113	107	100	40	88	103	139	126
10	40	72	72	70	110	100	140	88	58	75	84	81
11	100	112	110	62	106	100	40	44	56	67	69	67
12	100	76	83	64	111	117	60	64	54	90	77	77
13	80	92	105	94	117	112	120	188	90	127	86	96
14	60	96	112	192	119	114	180	96	60	52	79	75
Mean	111	113	130	110	156	148	76	70	59	65	81	77
S.E.	± 3.3	± 3.3	± 3.0	± 3.7	± 5.1	—	± 2.7	± 2.9	± 2.3	± 3.0	± 5.8	—
Sampling variance	154	154	291	192	361	—	105	115	73	122	—	—
Angaia variance	0	32	71	42	90	—	0	59	3	31	—	—

and the sampling variances did not differ significantly, but the angaia variances differed markedly but not in an orderly way.

From the estimates made for 100 random plants in tenancy B, calculations were made of the errors to be expected for samples of various sizes; these are summarised in Table X.

TABLE X.

Errors to be expected with samples of various sizes.

Standard error (S.E.)		Number of plant stations sampled per angaia						
		∞	100	50	20	10	5	1
S.E. (mean of single angaia)	..	2.22	2.26	2.30	2.42	2.60	2.92	4.79
„ as % of mean	..	28.5	29.0	29.5	31.0	33.3	37.2	61.6
S.E. (mean of 5 angaias)	..	0.99	1.01	1.03	1.08	1.16	1.31	2.14
„ as % of mean	..	12.8	13.0	13.2	13.9	14.9	16.8	27.5
S.E. (mean of 10 angaias)	..	0.70	0.72	0.73	0.76	0.82	0.92	1.52
„ as % of mean	..	9.0	9.2	9.3	9.8	10.5	11.9	19.5
S.E. (mean of 14 angaias)	..	0.59	0.60	0.62	0.65	0.69	0.78	1.28
„ as % of mean	..	7.6	7.8	7.9	8.3	8.9	10.0	16.4

It is clear that the number of angaias sampled per tenancy is more important than the number of plant holes sampled per angaia. For example, the percentage error of the mean for 10 plants in each of 14 angaias (140 plant holes) was 8.9; that for 100 plant holes in 10 angaias (1,000 plant holes) was 9.2.

From these findings a standard method of sampling a 10-feddan tenancy was adopted, consisting of a 500-leaf sample collected as five randomly selected leaves from each of 100 plant stations. These plant stations in early counts were selected at random in each of 10 angaias and later completely at random across the tenancy. Normally a team of five trained assistants was used, each counting the jassid nymphs on 100 leaves.

Study of the distribution of jassid nymphs within 90-feddan fields presented difficulties owing to the practice of watering tenancies A-D and E-I as two separate groups, so that when A-D was dry, E-I was wet, or vice versa, and wet fields cannot be traversed through the nature of the soil and danger of damage to cotton plants. Moreover, this watering practice affects the sowing dates, since tenancies A-D normally receive water first and are thus sown earlier than E-I. The possibility that sowing date might have an important effect on infestation was apparent from the counts given in Table XI, where there was a tendency in tenancy B, especially when big samples were taken, for infestations to be greater in the first than in the later angaias. Such a distribution might be a result of the sowing practice which, beginning on the side closest to the water channel, was shown by M. R. Norman (unpublished) to influence the distribution of fleabeetle (*Podagrica* spp.) in a cotton field.

Thus, between 7th and 9th October 1949, samples of 500 leaves from 100 plant stations of all dry tenancies in the administrative area of Rukn were examined. The results are summarised in Table XI.

TABLE XI.

Distribution of jassid infestation in 90-feddan cotton fields.

Tenancy	Tenancies sampled 7-9.x.49	Mean number of jassid nymphs per 100 leaves	Means
A	3	136	A—D 138 ± 8.9
B	3	162	
C	4	119	
D	4	136	
E	3	81	E—I 74 ± 3.9
F	3	69	
G	5	72	
H	3	83	
I	2	65	
Total and mean	30	102	

Such relationship between sowing date and early infestation was not confined to infestations within single cotton fields but occurred over wide areas. Thus, in 1953, when surveys were conducted in late September over an area of nearly 4,000 feddans of cotton, in spite of different dates of counts and the large natural variance over so big an area, the correlation of infestation with sowing date ($r = -0.4517$, significant at 5 per cent.) accounted for an important part of the variance of infestation in the area.

This relationship of infestation to sowing did not persist throughout the season. Thus, in the Rukn (1949) data, amongst 20 tenancies examined between 19th and 21st November, 10 were early sown (15th–16th August) and 10 were later sown (22nd–24th August). The mean infestations were 490 ± 46 and 488 ± 79 nymphs per 100 leaves, respectively.

To compare infestations in 90-feddan cotton Numbers it was, therefore, considered necessary to select tenancies at random and to increase replication sufficiently to accommodate variance due to possible ranges of sowing dates, or to select at random tenancies from each half Numbers (*viz.*, A–D, or E–I) and bulk the results.

Distribution of populations within Blocks.

On the S.G.B. estate all cultivable land is farmed within an 8-course rotation (see p. 199) and the remainder, which is never very extensive, is locally known as *balag* and supports scrub or *Eucalyptus* plantations. In each Block there are two to four houses, usually sited on an A tenancy, adjacent to a canal, the rest of which is cultivated as a garden. Within the Gezira rotation, cotton may thus fall adjacent to

- (i) irrigated gardens;
- (ii) irrigated sorghum (sown in July, before cotton);
- (iii) irrigated *Dolichos* beans (sown in October–November);
- (iv) fallow or resting land;
- (v) *balag* (scrub or *Eucalyptus* plantation);
- (vi) cotton.

Between 1949 and 1950, surveys were made of *E. lybica* on cotton in relation to these known sources of variance. *Dolichos* beans, being sown at a time when the population peak of *E. lybica* was being reached on cotton, were considered unlikely to affect numbers; cotton falling next to *balag* and other cotton (except within Numbers) did not occur often in the Blocks selected for survey. Accordingly, cotton Numbers were grouped as adjacent to gardens, *i.e.*, (i) above; sorghum, *i.e.*, (ii) above; or fallow, *i.e.*, (iii) and (iv) above. Counts were made on randomly selected tenancies from A–D and E–I of cotton Numbers by means of 500-leaf samples per tenancy and also by means of counts of nymphs on complete plant stations (plant holes) so that population estimates could be obtained.

In 1949, examination of counts made early in the season (September–early October), when populations were small, on seven Blocks distributed throughout the estate, revealed no obvious differences in infestation in relation to the situation of the fields; later in the season (mid-October) populations were much larger and so also were differences between situations within Blocks, but these were not consistent from Block to Block. In the southern Blocks a large and significant part of the variation in infestation was accounted for by variation in sowing date and growing conditions, as indicated by the number of leaves per plant, but this was not so in the more northerly Blocks. In 1950, counts made in three Blocks in the Gezira area, between the 15th and 30th September, showed that fields adjacent to irrigated areas (sorghum and gardens) supported significantly greater populations than did those adjacent to resting lands.

Whether these early differences persisted could not be decided because all cotton was sprayed early in October. They illustrate, however, that variation in density of populations within a Block may be in part a result of differences in proximity to sources of infestation, provided by irrigated gardens or other irrigated crops.

The colonisation of cotton.

Cotton sowing normally begins over most of the S.G.B. estate by the 12th August, and by the 30th August is complete over about 95 per cent. of the area. Germination takes about $3\frac{1}{2}$ days. Since development from the egg to the adult stage in *E. lybica* occupies 16–22 days, almost all nymphs discovered on cotton during the first two weeks of September must be derived from immigrant adults. Subsequently, adults might be immigrants to or bred within the cotton fields, and there was no simple method of distinguishing the two types. The hypothesis that peak populations on cotton in November–December are determined by the extent of destruction of nymphs on alternative host plants by rainstorms in July–August (Hanna, 1950) would imply that there are marked differences in the size of the initial infestation on cotton. Accordingly, between 1949 and 1951, detailed surveys were made of the incidence of nymphs of *E. lybica* on cotton in the Blocks where populations in gardens and on fallows had been studied earlier in the season. For the purpose of analysis the results (Table XII) were bulked under northern, central and southern localities.

TABLE XII.

Comparison of initial colonisation of cotton with July–August rainfall, Sudan Gezira.

A. Estimated mean population (in thousands per feddan) of nymphs of *E. lybica* in three localities between 10th and 20th September during three seasons (brackets show number of 10-feddan fields sampled).

Localities	1949	1950	1951
Northern	0.99 (20)	9.79 (44)	0.29 (12)
Central	6.60 (13)	12.43 (18)	4.30 (40)
Southern	1.46 (32)	7.70 (36)	7.20 (20)

B. Mean rainfall (mm.) in three localities during July and August of three seasons (brackets show number of rain gauges used in calculating mean rainfall).

Localities	1949	1950	1951
Northern	130 (8)	219 (7)	82 (2)
Central	116 (8)	275 (3)	160 (6)
Southern	165 (9)	313 (4)	306 (4)

In an analysis of the data in Table XII by calculating the co-variance between the September populations of *E. lybica* on cotton and the rains falling between July and August, the co-variance due to seasonal effects was eliminated and the correlation coefficient calculated from the residual co-variance had a value of +0.7130. Although this value is not significant for three degrees of freedom, it is large. It suggested that the greater the pre-sowing rainfall, the greater the initial colonisation of cotton, as was tentatively concluded from the surveys of populations on cotton and fallows.

In subsequent surveys, which were made annually between the 18th and 26th September, from 1950 to 1955, several Blocks were sampled in each of four

geographical divisions of the estate. Analysis of the counts in relation to the season's rain gave the results set out below:

Correlation coefficients for initial colonisation of cotton by *E. lybica*
(expressed as nymphs per 100 leaves) and rainfall (mm.).

	d.f.	x_1	x_2	x_3
Localities	2	0.9933***	0.9716**	0.5714
Seasons	4	0.7254*	0.7887*	0.6449
Residual	14	0.0197	0.4526*	-0.6701***

x_1 = Rainfall during July and August.

x_2 = Rainfall from 1st July to 15th August.

x_3 = Rainfall during September.

* $P=0.1$, ** $P=0.05$, *** $P=0.01$.

The apparent contradiction, given by the positive, though non-significant, correlations between infestation and September rains in the locality and seasons components, is resolved by the fact that these correlations are positively associated with their respective significant correlations of pre-sowing rainfall and infestation. This means that high pre-sowing rains tend to be followed by relatively high September rains, but, when considered in conjunction with the residual correlations, the effect of the high pre-sowing rains tends to override that of high September rain when and where the latter occurs.

Population changes on cotton.

Population changes on cotton in various Gezira Blocks during several seasons are shown in fig. 3 to illustrate the variety of curves of increase encountered. The data represent the mean numbers of nymphs of *E. lybica* recorded on a 500-leaf sample from each of several (6-40) tenancies in each Block. Since the increase in number of leaves per plant is linear between the time of germination and mid-October or later, the curves would not have been very different had populations per plant been plotted instead of leaf infestations (see fig. 4).

The feature common to these curves is the rapid, approximately exponential, increase during October and November of populations, which reach a peak in November or early December, followed by an equally sharp decline from which no recovery occurs. The pattern of increase is variable and the peak cannot be predicted from any preceding point on the curve either by analysis of data within Blocks or between Blocks in any season. Two examples are given:

Five tenancies in each of eight Numbers within a Block were sampled between the 7th and 16th October 1949, and again between the 3rd and 28th November, when populations were reaching their peak. The correlation coefficient describing the relation of the October to the November counts, calculated from the residual co-variances, after eliminating effects due to variability between Numbers and between tenancies, was 0.2122, which, for 27 degrees of freedom, is not significant.

In 1955, three tenancies in each of ten Gezira Blocks were sampled between the 29th September and 4th October and again between the 30th November and 26th December, when peak infestations were attained. The correlation coefficient of the residual co-variance, after eliminating effects due to variability between Blocks and between tenancies, was -0.4006, which for 17 degrees of freedom is of doubtful significance ($P=0.10$). Thus, there was a slight tendency for cotton with the highest infestation in late September to have the lowest peak, and vice versa.

The curve of increase thus seems to be determined by environmental factors operating during October or November or during both months, and not directly by any event prior to these months.

The final fall in population in December coincides with the commencement of

maturation of the crop, accompanied frequently by leaf shed. That it occurs independently of degree of infestation, equally on sprayed as on unsprayed cotton, suggests that it is causally related to crop maturation.

The effect of rainstorms.

Goodman & Toms (1956) illustrate the deleterious effect of heavy rainstorms on

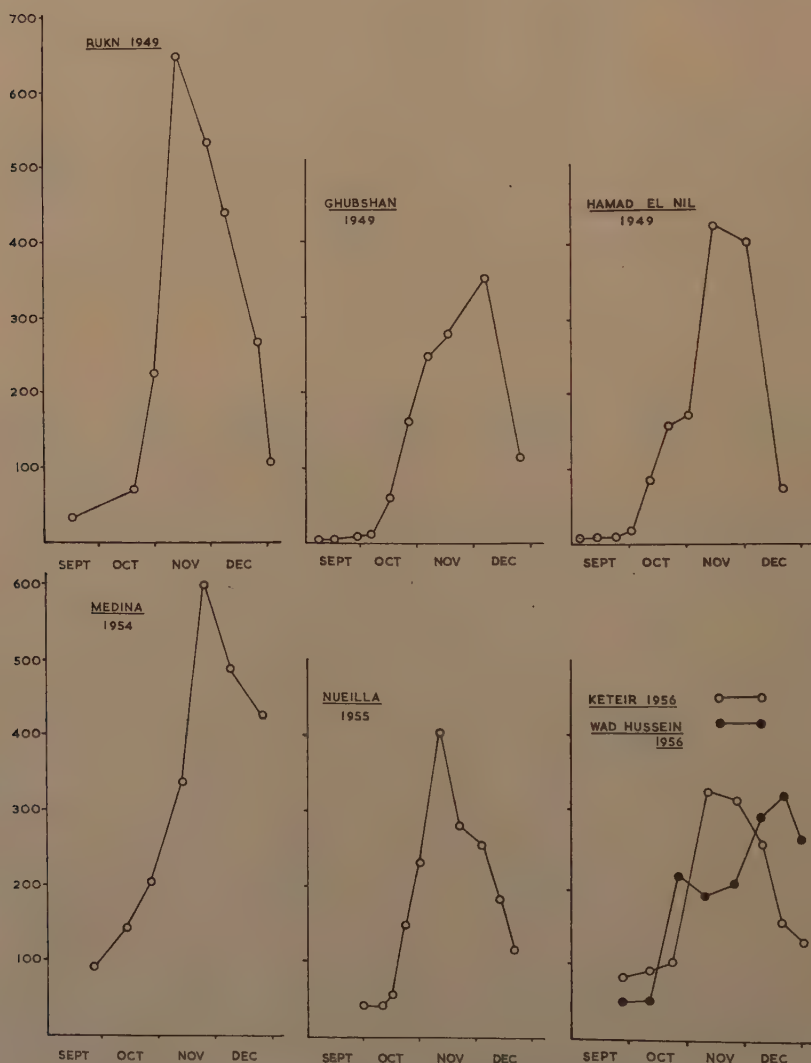


Fig. 3.—Curves of infestation densities (nymphs per 100 cotton leaves) of *E. lybica* in different seasons and places in the Sudan Gezira.

populations of *E. lybica* on cotton by examples of storms in September, and Hanna (1950) records the similar effect of a sandstorm in September but points out that such storms are unusual.

On the 10th September 1949, violent rain preceded by a sandstorm of exceptional severity swept across a defined track in northern Gezira. Some cotton fields were so badly damaged that they had to be largely re-sown. Infestations

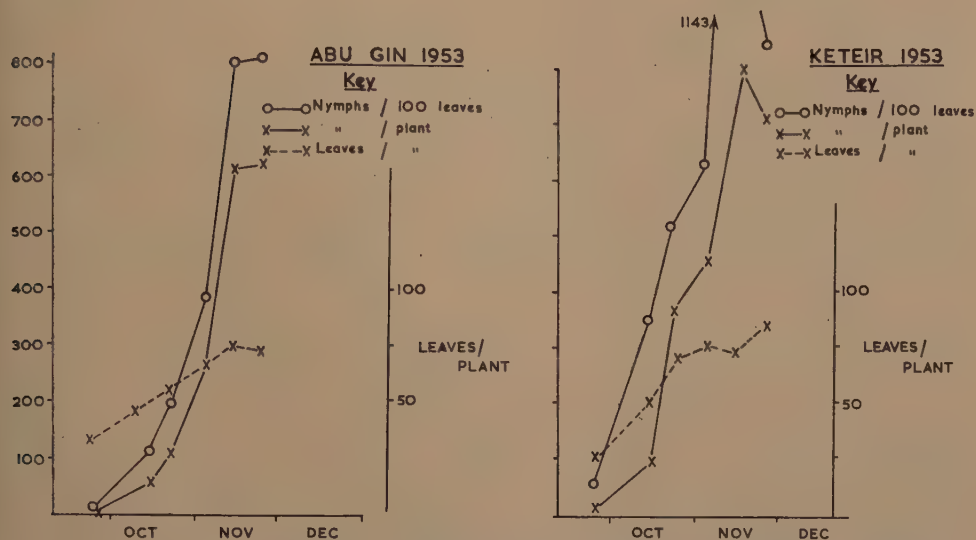


Fig. 4.—Curves of infestation densities (nymphs per 100 cotton leaves) and populations (nymphs per plant) in two Sudan Gezira localities.

were subsequently estimated on four of the worst damaged and four little-damaged tenancies, with the results given in Table XIII.

A less severe infestation of *E. lybica* was recorded on the storm-damaged cotton than on the undamaged cotton up to mid-November, i.e., for 10 weeks after

TABLE XIII.

The effect of a violent storm on 10.ix.49 on infestations of *E. lybica*.

Date	Nymphs per 100 leaves			P
	Cotton largely destroyed and resown	Cotton not damaged	S.E.	
10-12.ix	4.0	8.5	± 1.4	0.2
13-14.x	15.2	35.0	± 8.1	0.2
22-23.x	30.0	60.0	± 25.6	N.S.
1.xi	69.5	131.8	± 24.6	0.2
9.xi	175.0	287.2	± 73.7	N.S.
16-17.xi	120.8	192.0	± 19.7	0.2
24-29.xi	176.5	186.8	± 25.0	N.S.
12-15.xii	232.2	261.5	± 34.8	N.S.
24.xii	231.8	227.0	± 25.9	N.S.

the storm, although the difference was not significant. Subsequently the difference diminished, until by late December there was virtually no difference between the two sets of tenancies. Goodman & Toms likewise noted that by the end of November, the time of peak populations, there was no marked difference in the number of insects on cotton within and beyond the area affected by the storm of 26th September 1955.

The example is quoted to emphasise that populations of *E. lybica* on cotton in the Gezira can survive and recover from a catastrophe in which both they and their host are largely destroyed. The curve of increase shows that the effect of a single catastrophe was to delay the attainment, not to change the size, of the peak populations. For rainstorms to control numbers of *E. lybica* it is necessary for them to exert a continuous pressure against increase throughout at least an important period of the seasonal history. For this reason Hanna (1950) considered the incidence of rain falling in storms of 10 mm. and above. The same figures for jassid infestation and rain that were used by Hanna were extracted from Research Division records and subjected to an analysis of co-variance. The correlation coefficients (*r*) were as follows:

	Rainstorms 10mm. and above July—Aug.	Total rainfall July—Aug.	Degrees of freedom	<i>P</i>
Stations	— 1.0000	— 1.0000	0	—
Seasons	— 0.9497	— 0.9388	4	0.01
Residual	— 0.6732	— 0.5441	4	N.S.

It is inferred from these results that little information is lost if total rain, as opposed to storms, is examined. Also, in spite of the very highly significant negative correlation of rainfall (or rainstorms) and jassid numbers in respect of seasons, the absence of significant residual correlation suggests the possibility that the relationship might not be causal, as postulated by Hanna, but that jassid infestation and rainfall varied from season to season and both were inversely correlated with some uninvestigated factor.

Since 1949, most of the cotton of the Gezira Research Farm and all that of the outstations has been sprayed with DDT, but the data for 1949–1956 (excluding 1952) from all unsprayed plots at the G.R.F. has been examined for the relationship of mean initial populations (20–25th September) and peak populations with rainfall (Table XIV). The year 1952 is excluded because in that year unsprayed cotton was confined to small plots in insecticide experiments and gave untypical results (Joyce, 1956).

The analysis confirms the conclusions from the 1950–1955 surveys of the Gezira (p. 210) that initial infestation of cotton is greatest in years of good rains, although there is a suggestion that September rains have a deleterious effect. Peak infestations tended to be negatively correlated with initial infestation although not significantly so and, as Hanna found, peak infestations were negatively correlated with early rainfall. The greatest influence was exerted by the pre-sowing rains, but as these increased the initial infestations and reduced the peak ones, it is difficult to see how they could have the direct deleterious effect that has been postulated. By increasing the numbers of host-plants they could increase the sources and, therefore, the size of the initial infestation, but they were at the same time apparently positively correlated with the environmental resistance to increase that must operate between September and December.

The effect of variety, spacing and nitrogenous fertilisers.

Two main cotton varieties are grown in the Gezira, X1730A and Domains Sakel, which are largely confined to the southern and to the northern halves of the estate, respectively. Both varieties are grown in plots at the G.R.F. where observations are made each season on the effect on yield of variety and nitrogenous fertiliser at two levels and at close and wide spacing. The progress of jassid infestation on each treatment was observed for four years by means of bi-monthly

TABLE XIV.

Infestations of *E. lybica* on cotton at the Gezira Research Farm in relation to rainfall.

Year	Nymphs per 100 leaves		Rainfall (mm.)			
	Initial (20th-25th Sept.)	Peak (Nov.-Dec.)	July and first half Aug.	July and Aug.	Sept.	Total
	y_1	y_2	x_1	x_2	x_3	x_4
1949 ..	3	407	68	92	57	247
1950 ..	10	280	178	248	84	415
1951 ..	4	742	49	155	37	236
1953 ..	24	270	265	279	65	403
1954 ..	31	380	228	310	10	463
1955 ..	12	597	142	150	74	320
1956 ..	12	249	252	280	104	441
r_{xy_1} ..	-0.4107		0.7911	0.7971	-0.3908	0.7621
r_{xy_2} ..			-0.7681	-0.6295	-0.4991	-0.7482
$r_{y_1y_2}$..						

For 5 degrees of freedom and $P=0.05$, $r=0.7545$

TABLE XV.

The effect of variety, fertiliser and spacing on jassid infestation of cotton sprayed with DDT.

Mean number of nymphs of *E. lybica* per 100 leaves recorded over the period of assessment.

Details		1953/54	1954/55	1955/56	1956/57
Variety :	Domains Sakel	23	27	152	17
	X 1730A	25	30	188	19
Fertiliser :	Nil	22	26	155	16
	3N*	25	29	184	20
Spacing :	Close, 30 cm.	23	27	155	17
	Wide, 60 cm.	25	29	184	19
Mean		24	28	170	18
S.E. (Single plot)		2.3	6.4	25.4	2.0
S.E. (Treatment mean)		0.6	1.6	6.4	0.5
Least significant difference, $P=0.05$		1.8	4.7	19.0	1.5
" " " " $P=0.01$		2.4	6.4	26.0	2.0
Period of assessment		11.x-24.xi	13.x-3.xii	20.ix-2.i	15.ix-8.i
Number of counts		5	5	8	8

* 120 rotls N per feddan (1 rotl = 0.45 kg.)

TABLE XVI.
Infestation of *E. lybica* in relation to application of nitrogenous fertiliser, Gezira Research Farm, 1956.

Mean number of nymphs per 100 leaves.

Treatment	Pre DDT-spray		Post DDT-spray			
	23.ix.56	2.x.56	7.xi.56	26.xi.56	3.xii.56	10.i.57
ON	6.8	8.6	26.8	22.4	54.4	13.6
2N at sowing	6.6	16.2	25.2	20.0	39.2	11.2
2N at 1.xi.56	7.4	14.4	24.4	27.2	88.0	26.8

Mean per cent. of nitrogen in oven-dried leaves

Treatment	Pre DDT-spray		Post DDT-spray			
	23.viii.56	6.ix.56	19.ix.56	4.x.56	18.x.56	3.xi.56
ON	3.84	3.02	2.74	2.97	2.74	2.98
2N at sowing	4.03	3.53	3.92	3.54	3.45	3.28
2N at 1.xi.56	3.75	3.52	2.65	3.07	3.09	3.22

ON = No nitrogenous fertiliser.

2N = 80 rotls (1 rotl = 0.45 kg.) of nitrogenous fertiliser (as urea) per feddan.

counts of nymphs on 50 leaves per sub-plot taken at random from ten plant holes, also taken at random. Results given earlier in this paper (p. 205 onwards) indicate that part of the variance in jassid populations in relation to these factors was correlated with variance in leaf numbers. In the series of counts from the G.R.F. experiments given in Table XV, infestations are expressed as nymphs per 100 leaves, thus removing the variance due to leaves. It will be seen that small but significant differences exist indicating that plants that were fertilised with nitrogen, or of the variety X1730A, or widely spaced, supported higher infestations than plants respectively not fertilised, of the variety Domains Sakel, or closely spaced.

Increases in jassid infestation of a similar order were found to follow the application of nitrogenous fertiliser on a commercial scale (Joyce, 1959).

Further considerations of the effect of nitrogenous fertilisers.

The infestation by *E. lybica* was followed during 1956 in three of the eight treatments of an experiment in which nitrogenous fertiliser, as urea, was applied at successive dates during the first three-and-a-half months of plant growth. The primary objects of the experiment required the jassid infestation to be destroyed by DDT spray, which was applied on the 3rd October 1956. The mean infestations by nymphs of *E. lybica* in these treatments are shown in Table XVI together with the percentage of nitrogen (determined by the Kjeldahl method) found in leaf samples which were collected at approximately two-weekly intervals from the 23rd August to the 3rd December.

Before spraying, jassid infestation on all plots increased and the nitrogen content of leaves decreased. This increase in jassid infestation was related to the level of nitrogen recorded in the leaves in September by the equation

$$y = 7.98x - 78.11$$

where y = the increase in number of nymphs per 100 leaves from the 23rd September to 2nd October, and x = percentage of nitrogen per dry weight of leaf, transformed to degrees (angles = arc sin $\sqrt{\text{per cent.}}$). The standard error of the regression coefficient is ± 1.73 degrees ($P < .001$).

The relationship after spraying of jassid infestation to leaf nitrogen was investigated by an analysis of co-variance in which the components due to plots (replication), dates of sampling, treatments and interactions were eliminated, and

TABLE XVII.

Correlation coefficients of residual co-variances of jassid infestation (nymphs per 100 leaves) and per cent. of nitrogen in dry weight of leaves (expressed as degrees).

Degrees of freedom	At similar dates	% N 2 weeks previously	% N 4 weeks previously
24 ..	0.2133	0.2051	0.3790

the residual co-variance thus calculated. This process was conducted comparing jassid infestation with the leaf nitrogen at approximately the same dates, or at two or four weeks previously. The correlation coefficients of the residual co-variances are shown in Table XVII.

This analysis showed that the correlation was greater with the new generation of *E. lybica*, that is, the population found four weeks later, than with the population present on the leaf at the time of determining a particular nitrogen content.

The determination of leaf nitrogen ceased on the 3rd December and that of jassid infestations a month later, so that comparisons of levels of infestations with leaf-nitrogen content about a month earlier were possible for five post-spray occasions on each of the replicates of the experiment. This gave 45 comparisons. The components of co-variance between those factors attributable to replicates, dates of sampling, fertiliser treatments, and their interactions were calculated, so that an estimate of the residual co-variance was obtained. Regression coefficients could then be calculated describing the relation between jassid infestation and leaf nitrogen when the effects of treatments, or of dates, or of both treatments and dates, were eliminated. These regression coefficients are shown in Table XVIII.

TABLE XVIII.

Regressions of infestations of *E. lybica* (nymphs per 100 leaves) on the leaf nitrogen approximately one month earlier, expressed as a percentage of dry weight (transformed to degrees).

Components eliminated	Degrees of freedom	Regression coefficient	P
Treatment ..	41	11.24	0.01
Sampling date	39	6.00	0.05
Residual ..	15	5.18	0.05

The post-spray jassid infestation could be described by the equation

$$y = 5.18x - 35.3$$

when y = jassid nymphs per 100 leaves, x = per cent. of nitrogen in dry weight of leaves (transformed to degrees).

The standard error of the regression coefficient is ± 2.68 degrees ($P > 0.05$).

This regression was not significantly modified by treatments (i.e., by application of nitrogenous fertiliser), but was significantly increased as the sampling date progressed. Thus, though the nitrogen level in the leaf changed as the season progressed and as fertiliser was added, and jassid infestation increased erratically to a peak in mid-December and then declined, when these effects were eliminated, a significant part of the variance of jassid infestation could be explained by variance in the leaf-nitrogen level about a month previously.

Nitrogen uptake by the cotton plant appears to be of particular importance in the Sudan Gezira. Crowther (1941) studied growth records of cotton grown under standard conditions over twelve years and found that the earliest determination of the nitrogen content of cotton leaves, made within a day or two of germination, had the largest standard deviation per season as well as the highest values. Leaf nitrogen fluctuated during September, and thereafter slowly declined for the rest of the season. He found that a significant part (nearly 50 per cent.) of the variance in final yield was correlated with variance in percentage nitrogen in the leaves at the end of August and also until the end of September. Since Crowther (1944) had also shown that yields are highly correlated with pre-sowing rains, that is, those falling between the 1st July and the 15th August, attempts were made to determine the effect of these rains on the leaf nitrogen of young cotton plants. In conformity with his finding that the nitrate content of the top foot of Gezira soil was negatively correlated with pre-sowing rains, Jewitt (1953) showed that the percentage of leaf nitrogen was also negatively correlated with these rains over the three years during which he investigated the problem.

Although in subsequent years this correlation did not appear to hold, it suggested that, at least in some years, nitrogen uptake was unimpaired but its utilisation by the plant in growth was inadequate. In view of the apparent importance of nitrogen concentration in the leaf in relation to the development of jassid infestation, it seemed worthwhile examining the records of leaf nitrogen during the period when jassid infestations are important to plant growth, namely, September to December.

The seasonal changes in nitrogen content of cotton leaves has been followed since 1930 by analysis of the leaves of whole-plant samples at fortnightly intervals (Crowther, 1941). The mean nitrogen content of the leaves was calculated for each year from 1942 to 1956 from the fortnightly records of dry weight of leaves per plant and the amount of nitrogen per 100 g. dry leaf. Since the plots were sprayed with DDT annually from 1949 onwards, the examination was divided into pre- and post-spraying eras.

Taking the period 1942-1956 as a whole, there was no correlation between pre-sowing rains and leaf nitrogen concentration because during the pre-spraying era the correlation was positive ($r=0.0902$), while during the post-spray era it was negative ($r=-0.5240$), though not significant. During both eras the correlation between pre-sowing rain and nitrogen concentration in the samples collected between early September and the end of October was negative, that for the pre-spray era ($r=-0.3706$) being not significant and that for the post-spray era ($r=-0.7560$) being significant at 5 per cent. That is to say, after initial destruction of jassids by DDT spray, later conditions for jassid increase on cotton were most favourable in years of poor pre-sowing rains in so far as there was a tendency in such years for these to be a higher concentration of nitrogen in the leaf during September and October.

The addition of nitrogenous fertiliser did not significantly change this relationship, though the correlation between differences of nitrogen content and pre-sowing rainfall suggested that plants took up more nitrogen, when fertilised, after low pre-sowing rains than they did after high pre-sowing rains. The mean nitrogen content of leaves in seven samples taken from sprayed cotton between 6th September and 30th November over three seasons from plots receiving nitrogenous fertiliser at sowing (80 rotls N per feddan) as compared with no fertiliser is given in Table XIX.

TABLE XIX.

Nitrogen contents of leaves of fertilised and unfertilised cotton.

			Mean % N.		Pre-sowing rains (mm.)
			ON	2N	
1954	3.03	3.34	228
1955	3.20	3.85	142
1956	2.84	3.28	252

ON, nil; 2N, 80 rotls N per feddan.

Pre-spray infestations were too small to permit analysis of the effects of these seasonal changes in nitrogen concentration. Post-spray recovery of jassid populations was followed during three seasons (Table XX and fig. 5). Table XX shows

the mean nitrogen content of leaves from the 21st October (first post-spray sample) to 2nd December (approximately the date of peak jassid infestation) and the recorded peak infestation, together with pre-sowing rains (1st July–15th August).

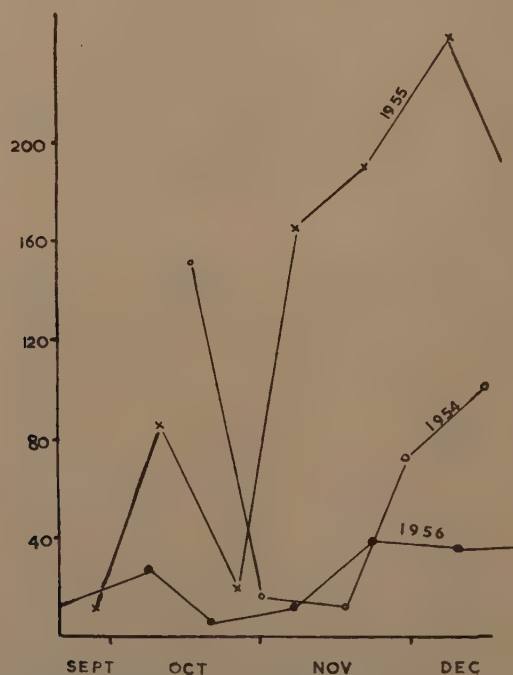


Fig. 5.—Recovery of infestation of *E. lybica* after mid-October spraying with DDT.

TABLE XX.

Crop-season levels of nitrogen content in cotton leaves and recovery of jassid infestations after spraying.

Year	Mean % N in leaves (Oct.—Dec.) x_1	Peak infestation y	Pre-sowing rainfall (mm.) x_2
1954	3.44	99	228
1955	3.64	240	142
1956	3.29	36	252

Correlation coefficients (after transformation of percentages to degrees) are as follows:

$$\begin{aligned}
 r_{yx_1} &= 0.9897 \\
 r_{yx_2} &= -0.9956 \\
 r_{x_1x_2} &= -0.9701
 \end{aligned}$$

With only one degree of freedom, none of these coefficients reached significance, but they were very high, and accord with a positive correlation of jassid infestation and nitrogen concentration in the leaf and a negative correlation of the latter with pre-sowing rains. They also support records made over four years

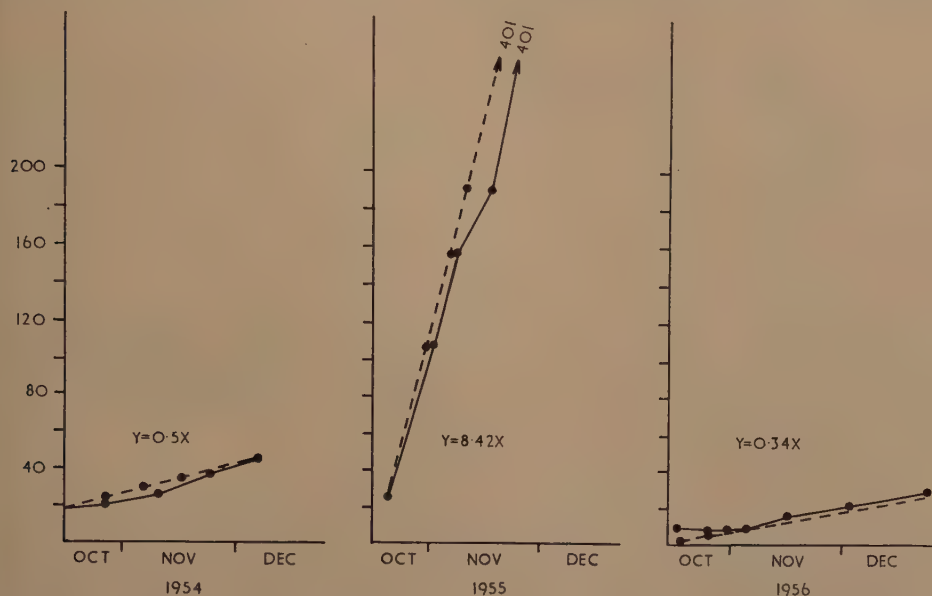


Fig. 6.—The recovery of infestation of *E. lybica* after mid-October spraying with DDT, 1954–1956 (Gezira Research Farm plots 16 and 17). Observed values represented by solid lines, values computed from the regressions by broken lines.

on a neighbouring plot. As fig. 6 suggests, on these plots the recorded post-spray resurgence may be described by the equation

$$y = ax$$

when y = nymphs of *E. lybica* per 100 leaves, x = number of days after spraying, and a = a constant representing rate of increase of nymphs (numbers per 100 leaves per day).

The rate of increase after spraying (mid-October to early December), concentration of leaf nitrogen (September–October) and pre-sowing rains (July–15th August) are shown in Table XXI.

TABLE XXI.

Rate of increase of *E. lybica* on cotton sprayed in mid-October.

Year	a (rate of increase)	x_1 (pre-sowing rains in mm.)	x_2 (% N in leaves)
1953	0.24	265	3.26
1954	0.50	228	3.24
1955	8.42	142	3.96
1956	0.34	252	3.19

An analysis of the regression of the rate of post-spray jassid increase on the mean September–October leaf nitrogen and pre-sowing rains gave the following:

Source of variance	Degrees of freedom	Sums of squares	Variance ratio (F)
Regression ..	2	48.6382	204.96*
Residual ..	1	0.1189	—
Total ..	3	48.7571	

* significant ($P=0.05$).

Thus, over 99.7 per cent. of the variation of the post-spray rate of jassid increase was explained by variation in September–October leaf nitrogen and pre-sowing rains; the value of r is 0.9989, which is significant ($P=0.05$ for one degree of freedom and two variables).

The regression equation describing this relationship was

$$a = 5.681x_2 - 0.0156x_1 - 54.541$$

where a = mean rate of jassid increase in nymphs per 100 leaves per day, x_1 = pre-sowing rains in mm. (from 1st July to 15th August), and x_2 = mean per cent. of nitrogen in dried leaves in September and October (transformed to degrees).

The quantities x_1 and x_2 , however, were highly correlated ($r = -0.9531$). When the contribution of that part of the variance of pre-sowing rains accounted for by leaf nitrogen was eliminated from the total variance explained by the regression, the direct effect of pre-sowing rains on post-spray jassid increase could be shown to be non-significant. The analysis of variance was:

Source of variance	Degrees of freedom	Sums of squares	Variance ratio
Variance due to $x_1 x_2$	1	48.3153	406.35*
Variance due to x_1	1	.3229	2.72
Total variance due to regression ..	2	48.6382	—
Residual	1	.1189	—
Total	3	48.7571	—

* significant ($P = 0.05$)

This suggests that the sequence of cause and effect is that pre-sowing rains affect the concentration of leaf nitrogen during September–October, which in turn affects the rate of increase of jassids from mid-October to early December.

Seasonal and site variation in nitrogen concentration in cotton leaves.

The Gezira observation plots described by Crowther (1944) consist of standard experiments comparing two varieties of cotton, two levels of nitrogenous fertiliser (0N and 3N) applied at sowing, and two spacings, in four localities. Those at the G.R.F. (central Gezira), Hag Abdulla (extreme south of the Gezira) and Turabi (northern Gezira), were established in 1938-39, and that at Abu Quta (north-west, on the Abdul Magid estate) in 1945-46. Each is cropped on a rotation of fallow-fallow-cotton. If the first three seasons, when virgin land was being cropped, are ignored, together with 1943, when records were incomplete, there are available, up to and including 1955-56, a total of 14 seasons when plant samples from three of these different localities have been analysed by a standard practice for the nitrogen content of various plant organs. Not all the eight treatments in the experiment were so sampled, however, and while the G.R.F. and Hag Abdulla plots compare the varieties X1370A and Domains Sakel, the Turabi plot compares two strains of Domains Sakel. Accordingly, an analysis was undertaken of the variance in leaf nitrogen concentration over 14 seasons at the three sites, in which the means of five samples taken during September and October from the 0N and 3N plots were considered, the varieties being ignored. The mean results are summarised in Table XXII.

TABLE XXII.

Mean nitrogen concentration in cotton leaves in 5 samples taken during September and October from 3 localities over 14 seasons, Sudan Gezira.

Locality	0N	3N	Mean
Hag Abdulla (South) ..	3.51	3.70	3.60
G. R. F. (Centre) ..	3.70	3.84	3.77
Turabi (North) ..	3.87	3.98	3.93
Mean	3.69	3.84	3.76

	Sites	Fertiliser treatment
S.E. (treatment means)	± 0.049	± 0.059
Least significant difference	0.14	0.17
	($P=0.05$)	
	0.18	0.22
	($P=0.01$)	

There were thus highly significant differences between the nitrogen concentration in leaves in the three localities, the values increasing from south to north, and being significantly increased by the addition of nitrogenous fertiliser. There were also highly significant seasonal differences, but no interactions between sites and seasons or sites and levels of fertiliser.

In order to investigate the possible effect of variety, the analysis was repeated using only data from the stations (G.R.F. and Hag Abdulla) where comparable varieties had been tested. There were no significant differences between the two varieties, in respect of nitrogen concentration in the leaves, either at the two sites or at the two levels of nitrogenous fertiliser.

The co-variance of the nitrogen concentration in leaves and the pre-sowing rains was investigated for all four stations (Hag Abdulla, G.R.F., Turabi and Abu Quta) for the six seasons (1951-52 to 1956-57) in which the plots had been

sprayed with DDT. The correlation coefficients (% leaf N on pre-sowing rains) were as follows:

		Degrees of freedom †	Significance (P)
Sites	-0.9397	2	<0.05
Seasons	-0.9552	4	0.01
Residual	+0.0584	14	N.S.
Total	-0.5842	22	0.01

In the 24 sets of comparisons (6 seasons \times 4 sites) a highly significant part of the variance in leaf nitrogen during September and October was negatively correlated with pre-sowing rainfall. When, however, the contributions associated with the two factors of sites and seasons were removed, the resulting (residual) correlation was not significant. That is to say, although leaf-nitrogen concentration was highly correlated with pre-sowing rains, there was no evidence that it was caused by this factor. On the contrary, it would appear that relationship between leaf nitrogen and pre-sowing rains is caused by some other factor, which itself is affected by seasonal and site variations in pre-sowing rains. There is no means of investigating from the data available what this other factor is.

Prediction of seasonal incidence of E. lybica in the Gezira.

Because of the apparent importance of the nitrogen concentration in the leaf in determining levels of *E. lybica* infestation on cotton, and because this factor was affected both by the application of nitrogenous fertiliser and by pre-sowing rains, an attempt was made to determine whether consideration of these two measurable factors could be used to predict jassid infestation. No systematic surveys of jassid populations over the whole of the S.G.B. estate had been made. Moreover, in 1950, the Gezira Board had adopted a policy of spraying all cotton in its estate, leaving unsprayed only a single 90-feddan Number in each Block, and not until 1956 were substantial areas again left unsprayed in each major geographical division. The unsprayed Number (90-feddan field) may be considered an inadequate sample of a cotton area of 2,000–10,000 feddans, particularly when considering a mobile insect, but leaf samples had been examined for jassid infestation, sometimes at regular intervals, sometimes at a date judged to represent that when peak infestations occurred, in a substantial number of Blocks in each season since 1949, and these provided the only data available. These records were, therefore, lumped together according to the geographical area of the Block to which they belonged, using the four geographical areas described by Snow & Taylor (1952). The estimates of pre-sowing rains used were those employed by the Research Division in estimating S.G.B. yields (Crowther, 1944) and represent the rain falling in the period 1st July–15th August, as recorded on gauges at each 'A' (Senior) house of each administrative Block. Fertiliser figures were obtained from the Gezira Board, and represent the total fertiliser (expressed as rotls of nitrogen) applied to the geographical division, divided by the total area (in feddans) of cotton grown in that division.

After effecting a log transformation of the figures for jassid infestation, an analysis of co-variance was carried out on infestation and rainfall, infestation and

† The degrees of freedom are derived as follows:

	Total d.f.	d.f. (for correlation coefficient)	d.f. (remainder)
4 Sites	3	1	2
6 Seasons	5	1	4
Error	15	1	14
Total	23	1	22

One degree of freedom is allocated for each correlation coefficient.

nitrogen and nitrogen and rainfall. The differences between the seasonal means for infestations, fertiliser level and rainfall were highly significant. The correlations between these variables are set out in Table XXIII.

TABLE XXIII.

Analysis of co-variance of peak jassid infestations (y), pre-sowing rains (x_1) and N fertiliser (x_2) in the S.G.B. estate, 1949-50 to 1956-57.

Source of variation	Degrees of freedom	$r_{x_1x_2}$	r_{yx_1}	r_{yx_2}	Value of r required for significance ($P=0.05$)
Sites ..	3	-0.9255	-0.9137	0.9999	0.9500
Seasons ..	7	0.4534	-0.3708	0.3607	0.7067
Residual	21	0.4853	0.0945	0.0527	0.4227
Total ...	31	0.3543	-0.3674	0.4099	0.3494

Thus, in the 32 sets of data (8 seasons \times 4 sites) peak jassid infestation was significantly correlated with pre-sowing rains (negative) and N fertiliser (positive). When co-variances due to site and seasonal effects were eliminated, there was no correlation in the residual variances. The data, therefore, provide no evidence that pre-sowing rains and nitrogenous fertiliser determine peak levels of jassid infestation, which are apparently affected by other factors, particularly those associated with sites, that are themselves correlated with pre-sowing rains. Since some of these factors connected with site (such as the fact that the north and south grow a different cotton variety) can be regarded as constant from season to season, it would seem reasonable, for the purpose of predicting jassid infestation, to take them into consideration. Accordingly, new correlation coefficients were calculated from the co-variance of the data when only seasonal effects were eliminated (Table XXIV).

TABLE XXIV.

Analysis of co-variance of peak jassid infestation (y), pre-sowing rains (x_1) and nitrogenous fertiliser (x_2) in the S.G.B. estate, after eliminating seasonal effects.

Source of variation	Degrees of freedom	$r_{x_1x_2}$	r_{yx_1}	r_{yx_2}	R	P
Sites + Error	24	-0.1263	-0.3837	+0.6177	0.6881	> 0.01

The relationship could be expressed by the equation

$$y = 2.144 - 0.0017x_1 + 0.0133x_2$$

where y = log of peak infestation of nymphs per 100 leaves, x_1 = rainfall (1st July-15th August), and x_2 = fertiliser (rotls N per feddan).

The standard errors of y , x_1 and x_2 are ± 0.156 , 0.0008 and 0.0036, respectively.

In fig. 7 the recorded infestation and that computed from this equation are plotted for the estate as a whole and for each geographical division separately. It will be seen that while there is a fair agreement between the two curves, there are, however, big seasonal variations not accounted for by the equation; these

may be caused by factors such as attacks by other pests, or by diseases, provided that these factors are not correlated themselves with rainfall or fertiliser applications. The data must, in any case, be accepted with reserve since they were not collected by a satisfactory sampling procedure. The general agreement of the equation derived from them with the conclusions reached from other data presented in earlier sections, however, justifies future study of this approach to the prediction of the size of jassid infestations.

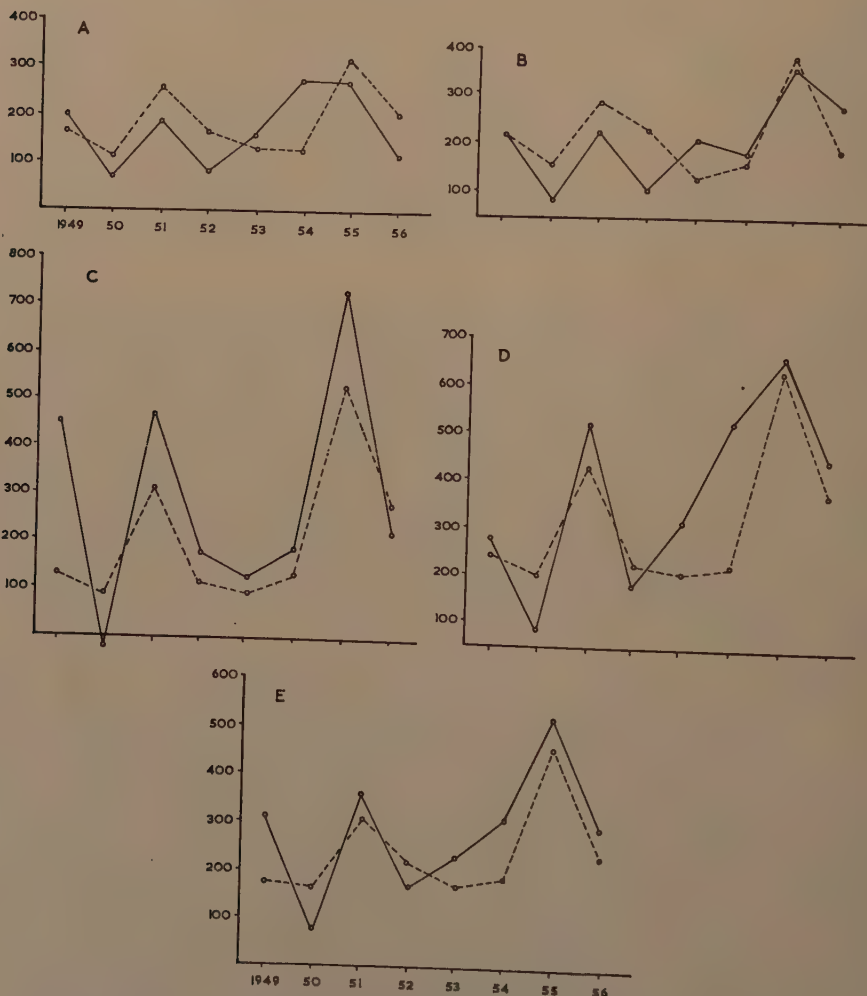


Fig. 7.—The recorded (solid lines) and computed (broken lines) peak infestations by nymphs of *E. lybica* (per 100 leaves) on cotton in the Sudan Gezira, 1949–1956. A, southern; B, central; C, north-central; D, northern; E, whole Gezira.

Discussion.

This account of some of the factors affecting the incidence of *E. lybica* on cotton in the Gezira differs substantially from that presented by other workers (Hanna, 1950; Cowland & Hanna, 1950), although their basic observations, namely, the increasing severity of jassid attack from south to north within the

Gezira, and the inverse relationship of this attack with pre-sowing rains, have been confirmed and extended.

Cowland & Hanna considered that irrigated gardens within the S.G.B. estate were the main source of the jassids that infest the cotton, and adduced as evidence a decrease in numbers of nymphs per cotton leaf as the distance from a garden increased. Data now presented show that, taking into account the extensiveness of fallows, and fields of sorghum, weed host-plants on such areas probably support far greater populations of jassids just before and during cotton germination than do gardens, and that such populations are greater in the south than in the north. Moreover, numerous perennial host-plants occur outside the Gezira, and it is impossible to delimit the sources of infestation or to estimate their relative importance, particularly as circumstantial evidence suggests that displacement over long distances may occur. Evidence is given that despite the demonstrably adverse effect of rainstorms on a given population of jassids, high rainfall nevertheless increases the total population of jassids on fallows by increasing the number of host-plants available for colonisation.

Surveys based on a reasonably accurate sampling method have shown that for at least a month after initial colonisation of cotton a substantial part of the variance in jassid infestation is correlated with sowing date. Because of the layout of the irrigation system in the standard Gezira Number, fields nearest to the canal (termed A fields) are usually sown earlier than those far away, and there is thus produced a gradient of jassid infestation from the A field to those farthest away. Since house gardens usually replace A fields, the succession of sowing dates down a cotton Number can provide an explanation of the gradients of infestation shown by Cowland & Hanna that is an alternative to the postulated dispersal from a source of infestation represented by the house garden.

The hypothesis that the size of the jassid infestation on cotton is adversely affected by heavy pre-sowing rains killing jassids by mud-splash (Hanna, 1950) implies that initial colonisation of cotton is greater in a year of light than of heavy pre-sowing rains. However, surveys over five seasons showed that, while September rains were associated with reduced infestations, initial colonisation of cotton was significantly greater in years of heavy than of light pre-sowing rains, a result consistent with the finding of larger populations in the fallows in seasons and places of high rainfall. Moreover, the peak infestation did not appear to be positively associated with the September one (which approximated to the initial colonisation). On the contrary, the higher initial infestations tended to be followed by the lower peaks.

The conclusion is inevitable that the peak size of the infestations on cotton is determined by the rate of breeding on cotton after the latter has been colonised. There is a tendency for this rate to be lowest when the level of initial colonisation is highest, and this level is in turn apparently determined by the number of fallow weeds available for breeding before cotton germination, which is highest in years of good pre-sowing rains. Thus the amount of rain before sowing increases the initial, but reduces the peak, infestations on cotton. It is difficult to understand how pre-sowing mud-splash could exert two such opposite effects.

Rate of population increase is determined by birth-rate and death-rate. Data presented in the present paper suggest that the rate of population increase is much influenced by the concentration of nitrogen in the cotton leaf, though they afford no evidence as to whether this acts by increasing the birth-rate or decreasing the death-rate.

A relationship between nitrogen content of host-plant and rate of reproduction of *Brevicoryne brassicae* (L.) was reported by Evans (1938), who also noted that the chemical composition of host-plants affected the length of larval and pupal stadia of *Pieris brassicae* (L.). Davidson (1925) recorded increased infestations of *Aphis rumicis* L. on crops receiving complete fertiliser treatments. Dahms

(1947) found that *Blissus leucopterus* (Say) had more eggs when fed on plants growing in solutions high in nitrogen or low in phosphorus, than on the same varieties low in nitrogen and high in phosphorus. Allen & Selman (1955) showed that egg-production of *Phaedon cochleariae* (F.) was much affected by the mineral composition of its leaf diet, a significant reduction occurring when leaves were deficient in nitrogen, phosphorus, potash or iron; they considered that reduced egg-production might be due simply to reduced protein intake. LeRoux (1954) found that populations of *Tetranychus bimaculatus* Harvey reared on cucumber were doubled when the initial concentration of nitrogen was doubled.

It seems reasonable to suppose that the association between the rate of multiplication of *E. lybica* and the concentration of nitrogen in the cotton leaf reflects a stimulation of the birth-rate, and as leaf-nitrogen concentration is negatively correlated with pre-sowing rains and increased by the application of nitrogenous fertiliser, it would follow that these two factors determine the birth-rate of *E. lybica* on cotton. It is not clear why the leaf-nitrogen content of irrigated cotton should tend to be higher for six weeks after sowing in a year of poor pre-sowing rains than in a year of good ones; there is no evidence that the relationship is causal, but it is consistent with Jewitt's (1953) finding of a negative relationship between the nitrate content during August and September of the top foot of Gezira soil and the 1st July–15th August rainfall. Crowther (1934, pp. 892, 893) has shown that the nitrogen concentration in cotton leaves during October was reduced by heavy watering, although the total uptake of nitrogen was not affected. Thus it is possible that poor pre-sowing rains, by allowing nitrate to accumulate in the top foot of the soil, enable young cotton plants to take up more nitrogen, but the off-take of nitrogen for plant growth, presumably through some other unfavourable factor correlated with pre-sowing rains, is reduced. The increased nitrogen in the leaf tissue is utilised by insect pests instead of by the plant.

No account has so far been taken of the possibility that pre-sowing rains are associated with an increased death-rate in some other way than through the physical effect of mud-splash, which is discounted as a major determining factor. Natural enemies might constitute such a link. Amongst the predators of *E. lybica* in the Gezira, Hanna (1950) lists *Coccinella rufescens* (Muls.) and *Exochomus melanocephalus* (Zoubh.) (cited as *E. nigromaculatus* (Goeze)), whose larvae and adults devour nymphs of *E. lybica*, and also *Chrysopa vulgaris* Schneider and spiders. Other predators since observed include other Coccinellids and Chrysopids, an Anthocorid bug (*Orius* sp.) and an unidentified species of thrips. Detailed observations by R. G. Allan (unpublished) on the effect of Coccinellids and Chrysopids showed that their effectiveness as predators of *E. lybica* on cotton on the G.R.F. was influenced by the presence or absence of other, preferred, hosts such as *Aphis gossypii* Glov. and *Bemisia tabaci* (Genadius). Moreover, in 1955, when *E. lybica* was abundant, these general predators were likewise numerous from October to December. On the other hand in 1956, when *E. lybica* infestation was extremely light on the G.R.F. cotton, these predators were rarely found until late November. It is unlikely that such predators can prevent an outbreak of *E. lybica*, but they may play a part in reducing numbers from peak levels.

On the other hand two species of *Anagrus* (MYMARIDAE) and a species of *Aphelopus* (DRYINIDAE) were found by G. Morcos (in Joyce, 1952f) to breed in the Gezira on eggs and nymphs, respectively, of *E. lybica*. R. G. Allan (unpublished) recorded no *Anagrus* in 1955, when jassid infestations were high and pre-sowing rains light, and many in 1956 when infestations were low and pre-sowing rains heavy, and suggested that these egg-parasites may play an important part in controlling the numbers of this jassid.

The nitrogen content of the cotton leaf, although demonstrably associated with the rate of breeding of jassid on cotton, is not necessarily the only controlling

factor that is correlated with pre-sowing rains. A study is necessary of the rôle of parasites, particularly egg-parasites, in determining death-rate, and of the nature and mode of action of any effect of pre-sowing rains on their numbers and activity.

Summary.

The Cicadellid, *Empoasca lybica* de Berg. is an important pest of cotton in the Sudan Gezira, where over 300,000 acres of cotton are grown annually under irrigation. Cotton is sown in mid-August, and the plants are uprooted and burnt the following May. The life-cycle of *E. lybica* from egg to gravid adult takes 16-24 days, and the adults live for up to 40 days. There is no diapause. During the 100 days from late August to early December when breeding on cotton is of economic importance, a single male and female could give rise to some 50,000 progeny.

During May to July, when crops are confined to irrigated gardens and river banks, *E. lybica* is widely distributed in such places and can be found also on tree hosts, which are numerous especially in the southern Gezira and along river banks. There is circumstantial evidence of displacement over long distances, and the great majority of catches of *E. lybica* in sticky traps were made before the increase in population on cotton that occurs from September onwards.

Of the 53 species of host-plants that have been recorded, only *Solanum dubium*, *Rhynchosia memnonia*, *Hibiscus* spp. and *Abutilon* spp. are of importance in the ecology of *E. lybica*. The first two especially are common weeds in fallows, which comprise more than half the land under rotation. Populations of *E. lybica* in Gezira fallows at the time of cotton germination tended to be greatest where pre-sowing rains (i.e., those falling from 1st July to 15th August) were highest. Correspondingly, initial infestation of cotton was highest in seasons and places receiving the most pre-sowing rains, although density of infestation in any place was affected by sowing date and proximity to irrigated fields and gardens which supported weed host-plants.

In order to develop a system of sampling for infestations of *E. lybica* in the cotton crop, the distribution of nymphs on cotton plants was examined. It was found that nymphs were most numerous in the leafiest zones of the plant and a random choice of leaves seemed an appropriate means of sampling for infestation. The distribution of nymphs within and between cotton fields was also investigated and a standard sampling procedure adopted.

Peak infestations on cotton could not be predicted from the level of initial colonisation, or from surveys a month later. Peak infestations were usually inversely related to the level of initial colonisation, especially when comparisons were made between seasons, as at the Gezira Research Farm. That is to say, high levels of initial infestation, which occurred in seasons of good pre-sowing rains, tended to be followed by low rates of increase, and in years of poor pre-sowing rains, initial infestations tended to be low and rates of increase high.

The relationship of these findings to those of Cowland & Hanna (1950) and Hanna (1950) are discussed; the hypothesis that pre-sowing mud-splash is a major factor controlling numbers of *E. lybica* in the Sudan Gezira is discounted, although it is accepted that this factor temporarily reduces populations.

The rate of increase of infestations of *E. lybica* was found to be positively correlated with the concentration of nitrogen recorded 2-4 weeks previously in the cotton leaf. This concentration affected not only the rate of increase of the initial colonisers, but also the rate of recovery of populations during November and December after spray-applications of DDT. The nitrogen concentration in the leaf was increased by nitrogenous fertiliser, with a corresponding increase in infestations of *E. lybica*. It was also found to be negatively correlated with pre-sowing rains, which, if low, prevent the nitrate in the top 12 in. of Gezira soil

being washed to lower levels, but the data presented provide no evidence that the relationship is causal.

It is concluded that localities and seasons of poor pre-sowing rains favour a high rate of increase of small populations of *E. lybica* because of high nitrogen concentration in cotton leaves during September and October. This tendency is augmented by application of nitrogenous fertiliser. A regression equation relating the peak infestations of *E. lybica* with pre-sowing rainfall and with nitrogenous fertiliser is given and the infestations computed from this are shown not to differ significantly from those recorded in the Gezira as a whole, and in the four main divisions of it separately, during the eight years 1949–1956.

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A MODIFIED LUMSDEN SUCTION-TRAP FOR BITING INSECTS.

By D. M. MINTER

*Division of Insect-borne Diseases, Medical Research Laboratory,
Kenya.*

(PLATE IV.)

While studying small Diptera (especially *Phlebotomus* and *Culicoides* species), in Kenya, a fan suction-trap was constructed for field use, based on the design of Lumsden (1958), but differing from it in several important particulars.

As it was intended to operate the trap in the bush far from a mains-electricity supply, and to station it on tree platforms as well as on the ground, it had to be constructed with the following considerations in mind: complete portability; battery operation; simple and robust construction, in sections for transport and ease of handling; simplicity of operation under arduous conditions, with minimum repair and maintenance; adaptable for bait animals of various sizes, from baboons to mice and lizards; able to collect insects alive and undamaged, particularly very small species.

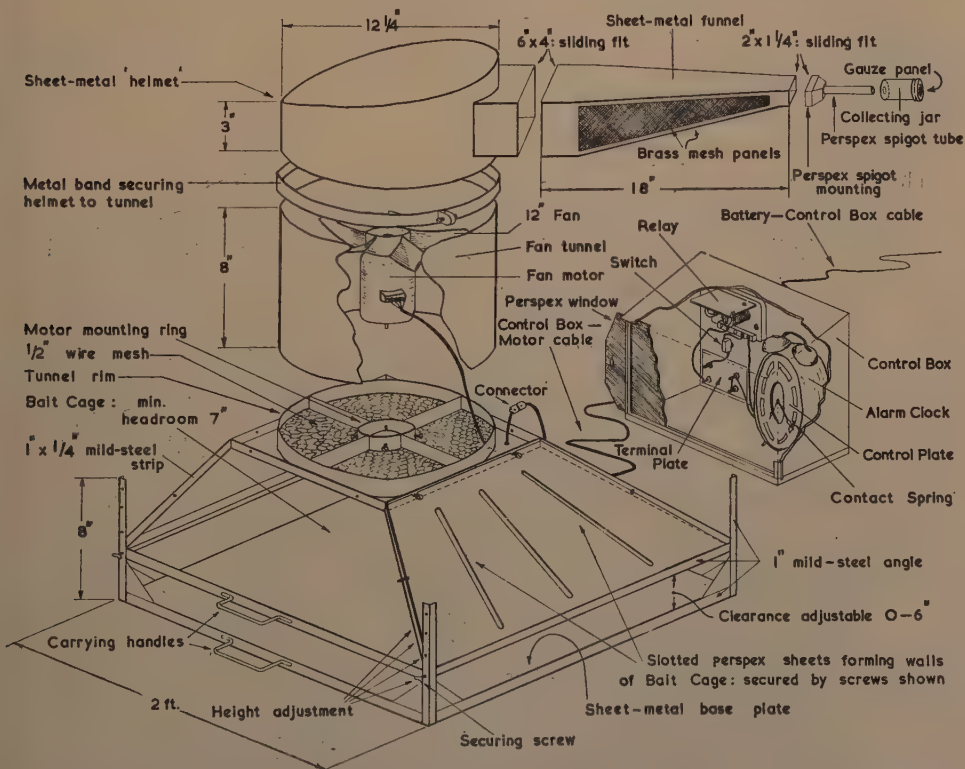


Fig. 1.—An exploded view of the trap assembled ready for use.

Three such traps proved very satisfactory when used almost continuously for over nine months; they caught large numbers of sandflies and midges. Mosquitos were collected in smaller numbers.

These traps were constructed by the writer, with the exception of the metal superstructure and fan housing. Anyone of average mechanical ability should find the trap easy to reproduce.

Structural details.

The fan and motor unit.

The fan is a five-bladed impeller of 12-in. diameter, manufactured by Vent-Axia Ltd., suitably bushed to fit the spindle of a 24-volt D.C. electric motor. (A government surplus teleprinter motor was used.) The fan and motor are mounted vertically in a sheet-metal housing above the cage portion of the trap, as shown in fig. 1.

Current is fed to the motor, *via* a time-switch mechanism, from a 12-volt heavy duty vehicle battery of 72-ampère-hour capacity. The motor when running takes approximately 3 amps., and the trap when operated on a routine of eight cycles per hour (in which the motor is running for a total of 20 minutes each hour) will function for 72 hours before the battery requires charging. In the field, two batteries were used alternately for 48 hours each, the one not in use being charged meanwhile. This is accomplished by means of a small 80-watt petrol-driven charging plant (government surplus), or by using the battery in a suitable vehicle if a charging plant is not available. When used nearer to the laboratory, batteries can be charged from the mains supply, using a conventional charging plant.

The 24-volt motor, operating on a 12-volt supply, produces a fan speed of about 2,000 r.p.m., and this gives adequate suction for small insects. In the motor used, speed is related directly to supply voltage; suction can therefore be enhanced if desired by an appropriate increase in voltage.

Superstructure and bait cage.

This consists of a number of removable parts (see fig. 1), listed below:

- (a) A sheet-metal base, two ft. square (one has been made three ft. square for larger animals), provided with welded angle-iron corner posts, into which the rest of the cage fits. Holes are drilled in the corner posts for screws to locate the upper parts of the cage at the required height above the base. A clearance from zero to six inches can be obtained. The height is selected primarily in accordance with the size of the bait animal, but in still-air conditions should be increased as far as possible. If gusty conditions are anticipated it should be reduced to zero or one inch; otherwise insects are likely to be swept out of the trap before they are taken up by the fan. Experience is the best guide in selecting the optimum opening for given circumstances.
- (b) The frame of the bait cage is made of four mild-steel strips welded to a square angle-iron base. Seven inches above the base is mounted the frame supporting the motor and the fan, which acts also as the roof of the bait cage. The motor fits within a sheet-metal ring, supported by four struts, and is secured (and centred if necessary) by means of four screws. The annular space outside the ring is closed off with $\frac{1}{2}$ -in. chicken wire to prevent unanaesthetised animals reaching up to the moving parts.

The open sides of the cage form a truncated pyramid which is fitted with removable sheets of Perspex, $\frac{1}{8}$ -in. thick. Each sheet is provided with $\frac{1}{2}$ -in. slots to admit visiting insects. The bait is thus clearly visible from all directions except immediately above. (Fig. 1.)

- (c) A sheet-metal tunnel, in which the fan operates, drops over a metal rim at the top of the cage, and allows a $\frac{1}{8}$ -in. clearance all round the fan blades.
- (d) Above the fan tunnel is a sheet-metal 'helmet' of the same diameter, secured in position by a metal band tightened with a screw. This helmet serves to deflect the air from the fan through a right angle into a gauze-sided funnel.
- (e) The funnel slides over the protruding lips of the helmet, is rectangular in section and 18 in. long, tapered as shown in the figure. It is fitted with panels of fine metal gauze on both sides and below, to allow dissipation of excess air pressure.
- (f) Over the funnel outlet is slid a Perspex structure carrying a spigot of Perspex tubing of $\frac{1}{2}$ -in. bore and some 5 in. long, which delivers insects into a collecting vessel.
- (g) This collecting vessel is a plastic laboratory screw-capped jar, 2 in. in diameter. The bottom of the jar is reinforced with a $\frac{1}{2}$ -in. block of Perspex, cemented to the outside, and is then drilled to the outside diameter of the spigot tube. The metal screw cap is cut out to receive a $1\frac{1}{2}$ -in. diameter piece of fine metal gauze, glued over the hole. The jar is pushed on to the spigot so that the mouth of the latter approaches to within $\frac{1}{4}$ – $\frac{1}{8}$ in. of the gauze in the cap. This allows the air stream to pass out, whilst insects are deflected back into the body of the jar; turbulence in the container is thus kept to a minimum.

In the case of small and delicate insects, it is necessary to change the jars hourly if insects are required alive for dissection or experiment subsequently.

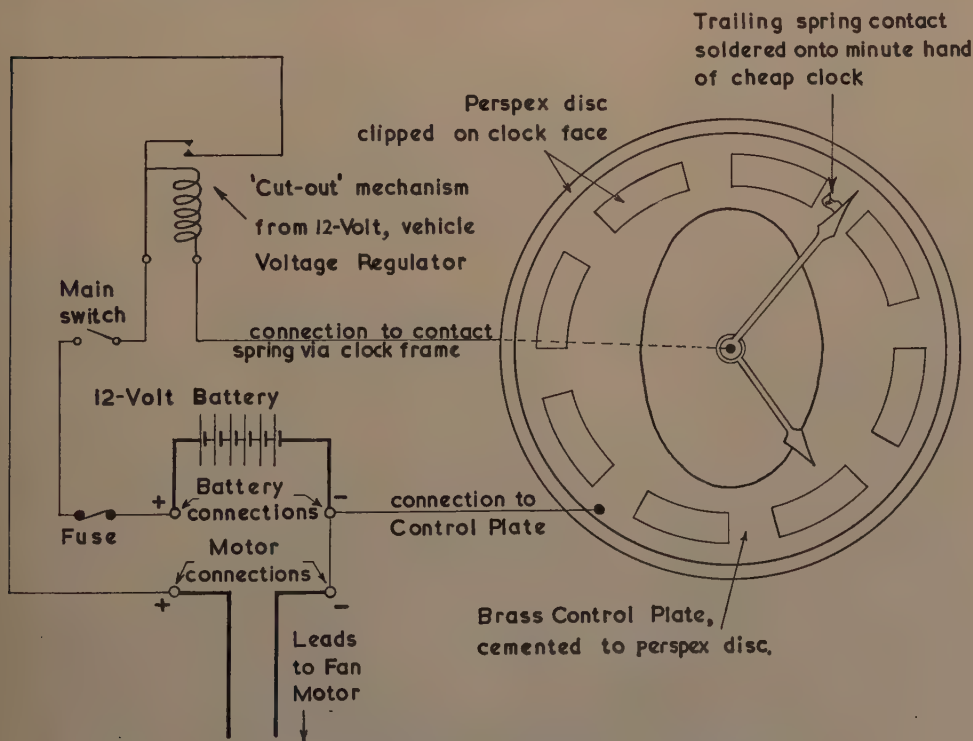


Fig. 2.—The electrical system and the control plate.

The time-switch mechanism.

This consists of a cheap clock with a disc of thin Perspex clipped over it in such a way that a piece of clock spring, soldered to the minute hand, trails across a sheet of thin brass, with appropriate sectors cut out, cemented to the Perspex with chloroform or dichloroethylene. The brass used for this purpose was 0.003-in. shimstock, sold in roll or packet form by motor dealers. The dissected segments are built up to, or very slightly above, the level of the surrounding brass with a cement of Perspex in chloroform. This is applied layer by layer; when finally dry, the composite disc is carefully ground down with fine sandpaper, and finished off by polishing with household metal polish. This avoids sparking by the trailing spring as contact is successively made and broken.

Current is fed to this simple mechanism *via* the cut-out portion of a 12-volt voltage regulator from a motor-car. Suitable regulators can usually be obtained without charge from garages, since only the cut-out circuit is required, which seems to burn out less frequently than the voltage regulator coil and its associated circuit.

The regulator used in the construction of this trap was Lucas model RB106/2, 12-volt. This, and the connections to and from it, are indicated schematically in fig. 2, which shows the complete electrical circuit, and again in fig. 3 in detail. If an intact and undamaged unit is used, it is preferable (but not essential) to cut the flexible wire to be seen (in the Lucas equipment) between the terminal block and the two coils. This wire is normally bright red in colour and lies alongside the varnished copper wire connecting the two coils. This removes from the circuit a small resistance coil which constitutes a small but unnecessary waste of current. No other alteration to the intact unit is needed.

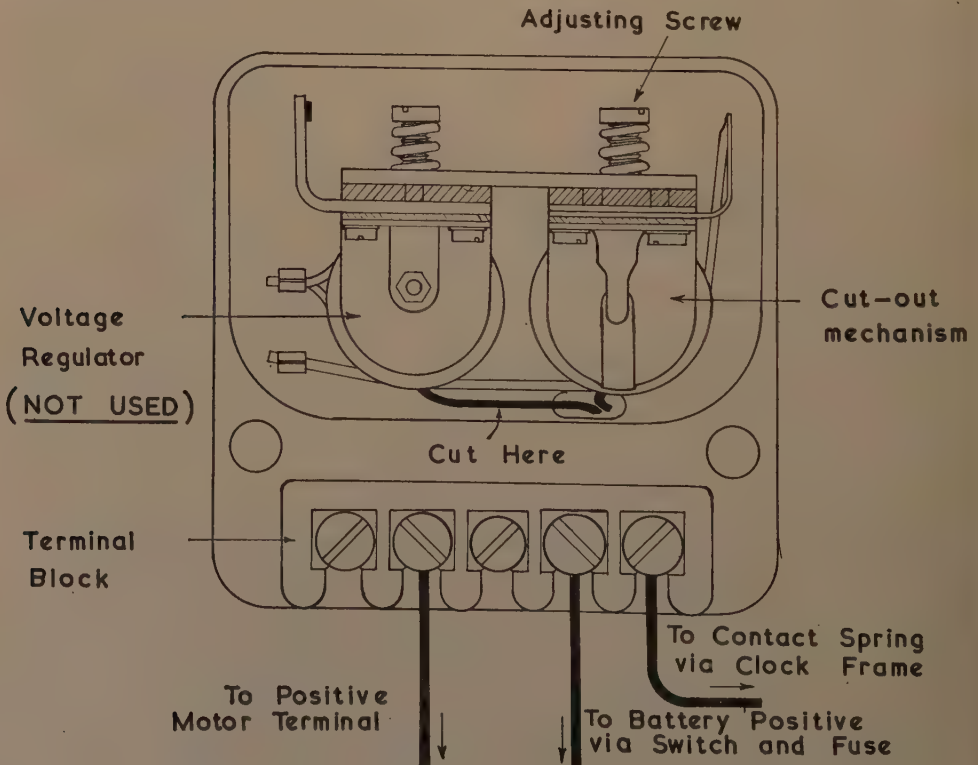


Fig. 3.—Plan view of Lucas cut-out mechanism and connections.

Due to the use of the cut-out, which functions as a magnetic relay, only a very low current (about 100 milliamps.), passes through the control-plate section of the circuit; this is sufficiently small to prevent damage to the plate and the trailing spring contact due to sparking or heating effects. The current to the control plate is carried by two wires; one soldered to the dissected brass sheet, the other connected to any convenient point on the clock frame in electrical continuity with the minute hand, and hence with the trailing spring contact. Details of the control plate are shown in fig. 2. The plate illustrated is that giving eight cycles per hour.

Clock and control plate, relay, fuse and switch, are mounted together in a weather-proof box: a terminal board is also incorporated in the back of the box, into which the cables from battery and motor are plugged. A hole is bored in the back of the box to enable the clock to be wound and set from outside, and the main on/off switch is also accessible in the same way. The box is completed by a sliding Perspex panel at the front.

Stages in the assembling of the trap are shown in Plate IV. (1) The bait-cage frame is fitted to the base. Motor and fan are mounted above; the Perspex sides of the cage are being fitted. Within the bait cage, a supply of food (for rodents to be kept there for some days) is shown. (2) A wild gerbil is swung by its tail as it is put into the bait cage. (3) The helmet is added. The time-switch box stands in front of the battery box; on top are two collecting jars, one already fitted on to the spigot mounting. Between the two jars is the gauze-sided funnel in end view. The plug-in connector between motor and time-switch is seen against the side of the trap. (4) Close-up of the trap fully assembled. (5) The trap operating beside a termite mound (right). On this occasion, the trap was baited with gerbils, and many sandflies were caught during several nights' operation.

The trap in operation.

As a result of Lumsden's (1958) findings in the case of mosquitos, a routine of $2\frac{1}{2}$ minutes on—5 minutes off was selected, giving eight cycles per hour. This has proved satisfactory also for smaller Diptera. It has been particularly effective for PSYCHODIDAE and CERATOPOGONIDAE. Mosquitos and larger insects have been trapped in smaller numbers, since the motor is operated at half its rated voltage, and thus at half its maximum speed. If one wished initially to trap larger species, a 12-volt motor would be used. In the present design, the choice of the 24-volt motor in conjunction with the 12-volt supply was deliberate. It was considered that half the full motor speed would be adequate for smaller Diptera: this has indeed proved to be the case. If, in future, larger insects are desired, it is a simple matter to increase the supply voltage; an additional 6- or 12-volt battery merely being connected in series with the present 12-volt one. The relay should still function effectively, since these are normally designed to allow for an overload somewhat in excess of 100 per cent.

It has been noticed that biting arthropods collected by the trap rarely have fresh blood in their stomachs. If insects were required to feed on bait animals it would be necessary to increase the 'off' period considerably by means of a suitable change of control plate. This might result in rather smaller numbers of insects being collected, since, in a longer interval, some might well succeed in escaping from the trap before being sucked into the container.

Various bait animals have been used as the attractant in the trap; baboons, monkeys, squirrels, gerbils, various other small mammals, and lizards. The trap has also been used, without the base, mounted directly on top of a conventional cage containing sentinel monkeys, on a tree platform in the forest near Nairobi. This has been in continual use for over nine months (as has another at Mombasa, on the Kenya coast). In addition to providing suitable material for dissection in the search for species that are vectors of disease, they have also contributed interesting data in relation to population dynamics.

Small animals of various species, either singly or in groups, have been housed for long periods in the bait cage, exactly as in conventional cages. For humane as well as practical reasons it is generally preferable to anaesthetise baboons, monkeys or other large animals. Repeat injections can easily be given if required without removing the animal.

Sandflies and midges for dissection are removed hourly from the collecting jar, briefly wetted in a 1 per cent. solution of detergent in normal saline, transferred to fresh saline, and stored at the bottom of a refrigerator or in a chilled vacuum flask. Such insects remain physiologically alive for 18 hours or more, showing peristalsis, and frequently a continuing heart-beat. This is very convenient in the field. Insects collected at night can be examined and dissected more easily the next day.

Experience with this trap has proved that it can be rapidly assembled, and equally speedily dismantled for stowage in a vehicle or hand carriage. The entire apparatus, together with the battery, can comfortably be transported by three men (Pl. IV, fig. 6).

The simplicity and robustness of construction enable the trap to be assembled and operated by subordinate staff with ease and confidence over long periods. Experience of many thousands of hours' operation under arduous conditions has demonstrated a remarkably high degree of mechanical and electrical reliability. The only complete break-down so far on record was due to a troop of marauding monkeys endeavouring to eat the plastic-covered cables. Requirements for routine maintenance have been found to be minimal, in spite of continual exposure to the vagaries of a tropical climate, and hundreds of miles of travelling over bad roads in the back of a Land-Rover. Providing the batteries are well looked after, it is necessary only to check the electrical connections occasionally, and to clean the brass control plate (and the trailing end of the spring contact) once a month.

Under conditions prevailing in Kenya, the total cost of each trap was about £25, exclusive of batteries. The greater part of this was incurred in labour charges for the construction of the steel frame and superstructure. The time-switch mechanism cost approximately Shs. 15/—, the motor Shs. 45/— and the fan assembly £6.

Summary.

The construction of a fully portable, animal-baited, fan suction-trap for biting insects is described, based on the principles employed by W. H. R. Lumsden in 1958. The robust form and simplicity of the trap, together with an independent power supply, make it ideal for use under field conditions. It has been found especially effective for small Diptera, notably *Phlebotomus*, *Culicoides* and related genera, in Kenya.

Acknowledgement.

My thanks are due to Mr. R. A. Forrest of the Royal College, Nairobi, for advice and help with electrical matters.

Reference.

LUMSDEN, W. H. R. (1958). A trap for insects biting small vertebrates.—*Nature, Lond.* **181** pp. 819–820.

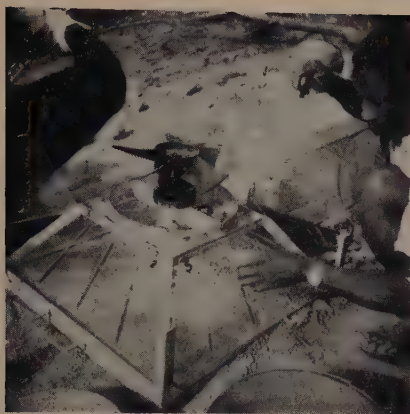


FIG. 1. Perspex sides of the bait cage being fitted.



FIG. 2. A gerbil being put into the cage.

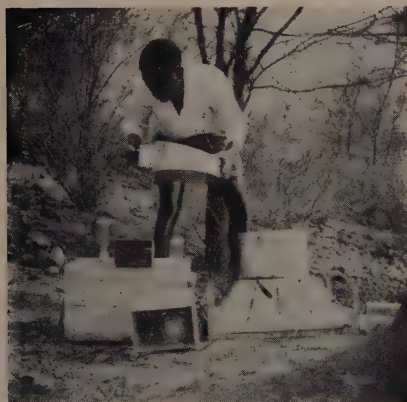


FIG. 3. Adding the helmet; various other parts are shown.



FIG. 4. Close-up of the trap fully assembled.



FIG. 5. Trap in operation beside a termite mound.



FIG. 6. Trap (left) and battery (right) carried by hand when dismantled.

PROBLEMS IN THE ASSESSMENT OF TSETSE POPULATIONS.

By K. R. S. MORRIS *

*Liberian Institute of the American Foundation
for Tropical Medicine.*

In making a survey of a species or genus of an animal there is the choice of the wide, general determination of distribution and range, or the detailed local study of it in relation to the natural features of its habitat. Each method has something to contribute, and a complete picture requires the application of both, but it is not always realised how greatly the operation of the general survey can be facilitated by knowledge gained in the detailed study and that the validity of the results may be seriously impaired by a lack of this knowledge.

At the start of the campaign against sleeping sickness in the Gold Coast †, in 1937, it was necessary to concentrate on detailed ecological studies in the most heavily infected areas in order to determine which species of *Glossina* were the significant vectors and to suggest, if possible, methods of control. Both of these objectives were attained (Morris, 1949). The intimate knowledge of the habits and habitats of the fly, occurring in the inland zone of savannah woodland (where the main epidemics lay), that was gained made it possible to plan a general tsetse survey.

The savannah woodland zone alone is a big area of 41,000 square miles in extent. Much of it was remote from roads and in a very primitive state of development. The problem of covering this country in the survey of an insect like the tsetse, which can be very elusive when not actually seeking a meal, would be almost impossible without a knowledge of the habitat where it can be found, the range of hosts which it will readily attack, and the periods of activity during which it will be seeking these hosts. The initial studies in the Gold Coast had shown the fallacies which could arise in too rapid a survey, mainly through not being in the right place, or using the right methods, at the time when the flies were most active. It was also realised that if the factors of habitat and activity could be understood, it would be possible to conduct the survey so as to obtain at least a relative idea of frequency instead of mere presence or absence. For this, a high degree of standardisation was essential, which must recognise not only factors in the biology of the tsetse but the equally important variables concerning the observers.

Survey in dry savannah country.

Factors of importance in the biology of the tsetse.

The survey of the savannah zone in the Gold Coast was lightened by the fact that only three species of *Glossina* were present, *G. palpalis* (R.-D.), *G. tachinoides* Westw. and *G. morsitans submorsitans* Newst. and that the habitat requirements of the first two, being specific riparian plant associations, were almost identical (Pomeroy & Morris, 1932; Morris, 1946). It was shown that these plant associations

* Now at Pathology Department, Makerere College Medical School, Kampala, Uganda.

† Since the work discussed here was carried out prior to the emergence, in 1957, of the Gold Coast as the independent state of Ghana (now the Republic of Ghana) within the British Commonwealth, it has been found convenient to refer to the territory, its subdivisions and localities as they were when the work was done.

were certain indicators of the presence of these two species throughout the inland savannah woodland zone and that during the dry season they never occurred elsewhere. The final proof of this relationship was given by the selective clearing of the specific fly-belt associations only, which resulted in the complete disappearance of the flies. In the course of these investigations a number of the African assistants became expert in the recognition of the more common trees of fly-belt and woodland. Thus, it was possible to organise mobile survey teams under trained leaders who were competent to spot at once a potential habitat and to record vegetation as well as species of *Glossina*.

Although the habitat of *G. morsitans* * differs, vegetationally, from that of the other two species, the territory it occupies is much the same, lying along the river valleys and drainage lines. Thus, fly-belts of this species would be traversed by the survey teams working along the rivers. Moreover, the habit of *G. morsitans* is to investigate and follow moving objects; and thus it was found that if this species was in the neighbourhood, it was always taken by survey teams during the course of their search for the riverine tsetse. The possibility of making accurate samples of the three species simultaneously was demonstrated as the result of ten years of continuous observation in the Kamba Valley (Morris, 1946, p. 237) by fly teams working on the standard routine designed for studying *G. palpalis* and *G. tachinoides*, which gave a clear picture of the appearance, spread and final reduction of *G. morsitans*.

The most difficult problem that has to be contended with in a survey is undoubtedly that imposed by the activity of the flies, which is subject to such a degree of variability as to give rise to most misleading results; the presence of the fly may even fail to be detected through search being made for too short a period or at a time when the insect is inactive. For example, a very experienced entomologist recorded the comparative scarcity of the riverine tsetse in the Northern Territories of the Gold Coast because he was able to catch *G. palpalis* and *G. tachinoides* at only seven places during a day's run of 200 miles (Nash, 1948). In point of fact, his route traversed fly-belts of these species at 23 different points. Such a startling error arose because it is quite impossible for one observer to be present at so many widely separated places during the comparatively short period of daily activity characteristic of these species. The need for prolonged search to detect tsetse at low densities or at times of reduced activity is a point of the greatest importance in survey which is evidently not always appreciated.

Studies of the diurnal activity of *G. palpalis* and *G. tachinoides* on the Volta river near Lawra were conducted in 1947 by making series of hourly observations from daybreak to sunset. The flies captured were liberated after being counted and recorded at the end of each hour, so that each series of observations could be continued for two to three weeks without there being a drain on the number of the flies, and the catches could most nearly represent their activity. The observations were repeated at different seasons for a year. There was a great variability in the activity of both these species throughout the day and from one day to another; and although a daily activity rhythm could be traced, it was easily upset by weather and varied greatly for different seasons. Since the difference between catches at times of high and of low activity can be extreme and since it was impossible to evaluate any correction factor, an hourly unit of catching was found statistically valueless. With standardisation as an essential condition in the formulation of the survey technique, a 'day' was taken as the minimum unit which would tap the activity peak irrespective of the time it occurred. This varied from the morning hours, during the hottest period of the year, to shortly after midday at the time of the cold harmattan wind in the dry season. So a standard 'day' from 7.00

* Throughout this paper the subspecies *submorsitans* Newst. is implied when reference is made to *G. morsitans*.

a.m. to 3.00 p.m. met the biological requirements as well as being convenient to the organisation.

Even a day's visit may fail to show tsetse when they are present in very small numbers, and a single day's catch is quite unreliable as a measure of frequency. An example is provided by an analysis of the data collected during eight years of continuous observation in a Volta river fly-belt where *G. palpalis* was scarce and *G. tachinoides* abundant. A team of four boys worked for 93 months for periods of five to six consecutive days each month. In all these periods, *G. palpalis* was only taken on 62 days, and never more than one example on a day. Each fly was a different one because those caught were killed. The reliability of searches continuing for one to six days in revealing the presence of this species during these observations is illustrated in Table I; the degree of reliability of a search for any period less than six days has been measured by the catch for the former period as a percentage of that for the latter.

TABLE I.

The reliability of different lengths of search by fly-boys in sampling *G. palpalis* at low densities.

	No. of days' search					
	1	2	3	4	5	6
No. of times <i>G. palpalis</i> appeared . .	26	37	47	56	61	62
Degree of reliability of searches (catch as % of total catch)	42	60	76	90	98	—

Comparable results have been obtained from other series of observations and show that when tsetse are at low densities, a single day's visit can miss up to 60 per cent. of the occasions when subsequent search has shown the fly to be present.

These studies showed that a minimum of four full days' catching, of eight hours each day, was necessary to ensure approximately 90 per cent. reliability in sampling and that even five or six days might not be sufficient to establish presence or absence of the fly when it is present at low densities. In the rapid survey procedure which was adopted in the Gold Coast, a standard of four full days' searching at each place was established as the minimum which would ensure that the survey was representative without being unduly prolonged. In this way, an estimate of frequency was obtained in well-represented fly communities and the chances of missing the occasional fly were greatly reduced.

The rôle of the observers.

It might be thought that the use of local, largely uneducated African boys for sampling would introduce variables even more difficult to overcome than those due to the behaviour of the tsetse. In fact, the rural African is a most competent observer; and with careful selection and training, teams were obtained capable of working with a high degree of consistency.

The most important point in the organisation of the teams was the separation of the function of catching from that of recording. The fly-boy (usually illiterate, but trained to a high standard of efficiency) concentrated on locating and catching tsetse without any of the interruptions caused by recording the catch, time keeping, mapping, etc., which were the duties of a literate and more highly-graded Recorder. Recorders were responsible for teams of two to four fly-boys and were given an itinerary which often kept them away for several months, working under very

arduous conditions. These leaders thus had to be men of great character and experience. An organisation of this nature made maximum use of literates, always a bottleneck in underdeveloped lands, but ensured that the full attention of the fly-boys could be concentrated on catching tsetse.

A study of the size of teams, made in a 12-month experiment with teams of one, two and four boys working in daily rotation in fly-belts of different densities, led to the following conclusions. At low densities, one boy catches on the average as many tsetse as do two or even four boys; it is only at high densities that the catch becomes proportionate to the number of catchers. In the gradation between these extremes, the point at which the size of catch becomes related to the number of catchers is reached only when the number of flies appearing at one time approximates to or exceeds the maximum catching capacity of the fly-boys. This maximum is a very variable quantity, being influenced by the species, sex and state of hunger of the flies. For example, with an elusive species and one with a host preference other than man, such as *G. morsitans*, the boys' catching capacity is numerically lower than with one which is easily caught, such as *G. tachinoides*, or a species which comes readily to man, as, for instance, *G. palpalis*. Males are more easily caught than female flies; the hungry fly is far easier to catch than the non-hungry. In fact, with the elusive *G. morsitans*, an expert boy may spend several minutes in the capture of one non-hungry individual whereas he might take two or three flies from a hungry swarm with one sweep of the net. Standardisation to the single fly-boy unit thus becomes a matter of great complexity if the catches have been made by teams of differing sizes and under different conditions. Approximate factors were worked out for standardising 4-boy, 2-boy and 1-boy catches, but the figures were only comparable within certain limits. It is more accurate and much simpler to undertake comparative work always with teams of the same size. Since the number of days is far more important than the number of boys for truly representative sampling, the teams were kept small, so that the personnel could be spread over a number of localities at once and yet each team could spend adequate time at each place.

Organisation of a survey.

In utilising the foregoing information for the development of a standardised survey technique, it was first necessary to decide whether to adopt the orthodox 'fly-round' procedure of East Africa and Nigeria, in which a moving team follows a carefully laid out route and measures the catch per unit distance, or whether a 'picket' method would not be better, with a more-or-less stationary team confining its attention during the whole period of observation to the neighbourhood of an easily definable point in a fly-belt, such as a water-hole or road crossing, and measuring the catch by units of time. While the fly-round is an invaluable technique in ecological studies of the game tsetse, there were overwhelming reasons in favour of the stationary picket fly posts for a survey in which the riverine *G. palpalis* and *G. tachinoides* were particularly concerned. In the first place, it was manifestly impossible to lay out standard fly-rounds, the catches from which would have any claim to comparability, when different Recorders were visiting a great many localities with widely-varied and as yet unknown topography and vegetation. In the picket system the instructions to Recorders were to locate the fly-belts neighbouring a village and to set the team to catch for a stated period at the nearest water-hole or road crossing, a perfectly uniform procedure and easy to follow. Further, experience had shown that the riverine tsetse came more readily to a stationary than to a moving object, and it was usual for the boys to get more flies after sitting or standing for a short time than straight away. Moreover, *G. palpalis* and *G. tachinoides* are constantly moving along the river banks and within the fly-belt, and as long as the flies remain active they will continue to come to the boys without the latter having to search more than a few yards.

A very serious disadvantage of the moving fly-round is the question of activity. With such a pronounced variability in the activity rhythm, from day to day and from one season to another, the stationary team has the advantage of being on the spot for two-thirds of the hours of daylight to tap this activity period when it occurs. The fly-round team, traversing different places at different times and catching during only three to four hours a day, might miss the maximum activity entirely on some occasions and hit it at others. For the results to be uniformly representative, it would be necessary to vary the time of the fly-round according to the time of activity, provided of course this were known for the season and locality. Such a procedure negates the principle of a standard sampling technique, the basis of uniformity in a survey.

A comparison of the two methods, at a time of year when the activity of the flies was restricted, showed that moving teams working on the fly-round technique caught only one-fifth of the number of *G. palpalis* and *G. tachinoides* that were captured by stationary teams working in the same fly-belt and for the same length of time. Teams consisted of two boys each; and it is interesting to note that in two 'animal' traps set in the same fly-belt on the river bank, the catches were double those of the stationary team during a trial lasting for two months. Similar rates of comparison were found in recent work in Liberia (Morris, in press). This demonstrates the advantage of being on the spot all the time and shows the way in which continuously high catches of the riverine tsetse can be made in one place by perfectly immobile objects.

The routine survey procedure was as follows. Teams of a Recorder or Field Assistant and two fly-boys were sent out, usually with bicycles, either to areas of special importance or on transect surveys, having been given a map and a planned itinerary which would take them two to three months to complete. Their objective was to locate, study and describe all the rivers on their itinerary on which recognisable fly-belts of *G. palpalis* and *G. tachinoides* were found. At each river, the Recorder arranged a 4-day catching period and entered a description of the river and locality on the standard questionnaire form, the same page being used for the records of the four days' catching. Catching was always at a water-hole or a road or path crossing, whichever was nearest to a village. Four days were spent at each place, whether tsetse were caught or not. The fly-boys were free to move about within the fly-belt in order to find the most advantageous spots for getting their flies, but did most of their catching in quite a small area. Thus, they could work the standard 8-hour day without undue fatigue or loss of interest, which is impossible on the walking fly-round procedure. The catches made in this way concerned mainly *G. palpalis* and *G. tachinoides*; but when *G. morsitans* was encountered in the neighbourhood, special attention was paid to it and separate notes were made of the numbers and time of search. It was thus possible to establish the distribution and approximate incidence of the three species in one operation.

The limitations of fly-boys for sampling.

The general survey was started in 1943, but was conducted for the six months of the rains each year only, because of the urgent need to keep all staff on sleeping sickness control during the dry season. Four to six survey teams were put into the field each year. In five years all but the remoter, uninhabited parts of the savannah woodland zone had been covered sufficiently for the distribution of the three species of tsetse to be mapped with a degree of accuracy which needed little subsequent modification.

Survey was then extended southward into the zone of dense bush and open glades transitional between true savannah woodland and rain-forest, into the forest itself, and later into the strip of savannah and transition forest along the coast. In these zones, *G. palpalis* and species of the group of *G. fusca* (Wlk.) occur throughout, *G. longipalpis* Wied. and *G. medicorum* Aust. occur in the transition

zone alone and *G. pallicera* Big. in the forest. Conditions of habitat and of climate were very different from those in the inland savannah and, with the exception of *G. longipalpis*, little was known of the habits of these species except that they were shy and difficult to capture. The demands of the northern sleeping-sickness problems did not permit any further investigations on these rarer forms at the time, and the survey was continued, using the standard procedure to ensure comparability with the surveys previously carried out; and because adherence to a known routine minimises mistakes when a team consisting entirely of Africans is employed.

It soon became apparent that these so-called rarer species of tsetse were, in fact, widely distributed, but they were nearly always taken singly and very occasionally only were two or three individuals taken from one locality. *G. nigrofusca* Newst. was the most commonly encountered species but, on many occasions, it was not recorded at all in places where a growing familiarity with this fly led the boys to expect its presence and where the more forthcoming *G. palpalis* was taken in numbers. The wide distribution and frequency of appearance of *G. nigrofusca* suggested that it was really a common tsetse, yet it was difficult to visualise the persistence of fly populations at the low incidence shown by the numbers captured. In fact, the evidence suggested that the sampling method was inadequate for this species. This supposition was confirmed by the discovery that, by taking a horse as bait and searching at dusk, *G. nigrofusca* could be caught in small numbers on any evening in the forest edges around Kumasi, where fly-boys on the standard daily routine had been working for months without revealing the presence of this species.

G. longipalpis is another man-shy species which is difficult to survey because it does not attack man readily. In 1931, the writer developed a method for its capture, by careful examination of the undergrowth, more flies being caught thus than came to feed on the fly-boys (Morris, 1934). Recently, Nash & Davey (1950) have shown that a similar method can be used for detecting *G. fusca* and *G. medicorum* as well as *G. longipalpis* in mixed deciduous forest in southern Nigeria.

A simple form of trap had been developed, in 1940, which was very effective in taking *G. palpalis* and *G. tachinoides* under the dry conditions of the inland savannah (Morris & Morris, 1949). Under certain conditions, particularly those unfavourable to activity on the part of the fly and with tsetse at low densities, the traps were a more effective means of sampling than were boys. Consequently, it was decided to experiment with these traps as an alternative to fly-boys in the forest. Experiments were started in 1950, in a project on the control of *G. palpalis* around Kumasi, and, a year later, similar investigations were made on the sea coast in transition forest with *G. longipalpis* as the most important species present.

Survey in moist forest and thicket.

Sampling G. palpalis and G. nigrofusca in rain-forest.

The great differences between the Ashanti rain-forest and the inland savannah woodland raised two main difficulties in conducting the survey. First, the additional species of tsetse present, and the fact that even the familiar *G. palpalis*, when in the forest, might have different habits. Secondly, the vast extent of evergreen vegetation and the more equable and humid climate afforded an enormous area of potential habitat, all of which had to be searched until a more exact knowledge was acquired of the habitat requirements for each species.

The initial trials with traps, undertaken to evaluate the effect on *G. palpalis* of bush clearing around Kumasi, proved that this type of trap was as effective in the deciduous rain-forest as in the dry savannah and gave indices of fly reduction comparable to those obtained by a team of fly-boys working the same area (Nowosielski-Slepowron, 1953). But the traps showed something more. After clearing was finished, the regular appearances of small numbers of tsetse in certain

of the traps, 20 of which had been spaced at intervals along approximately a mile of valley, led to the detection and elimination of two small pockets of flies which had been overlooked in adjacent uncleared bush. Herein lies the virtue of the entirely mechanical process of trapping. Irregularities in the captures from any individual traps are quickly apparent and draw attention to some event in their immediate vicinity.

The Tsetse Control Department was then asked to survey and advise on the protection of two important groups of buildings, the Prempreh College and the temporary hospital, standing in an area of open ground approximately a mile long by half a mile wide, surrounded by tall forest on one side and low, regenerating forest on the other (fig. 1). This gave a periphery of about three miles of potential

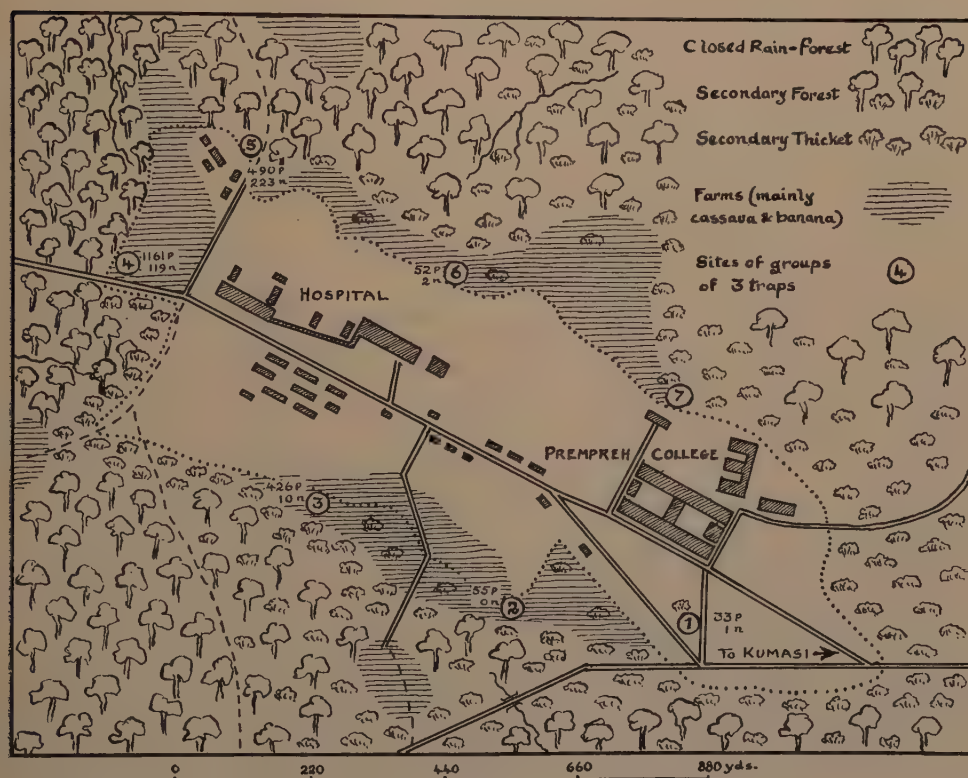


Fig. 1.—The distribution of *Glossina palpalis* and *G. nigrofusca* as shown by traps in an area for development in deciduous rain-forest close to Kumasi, Gold Coast. The figures by each trapping site give the numbers of *G. palpalis* (p) and *G. nigrofusca* (n) caught in each group of 3 traps in 18 weeks, April-August 1950.

habitat. Within the open area some cassava farms and dense patches of bush also offered suspect haunts of *G. palpalis*. In other words, a large area had to be searched. There were frequent complaints of tsetse attack from residents. To facilitate the initiation of protective measures, it was necessary to find out if the flies had any particular distribution pattern in the surrounding forest, if they lived, indeed, within the College grounds, and if they followed any regular routes. Information was desirable over a sufficient period to cover both wet- and dry-season conditions. It was decided therefore to make use of traps in this survey; the

TABLE II.

The frequency and distribution of *Glossina palpalis* and *G. nigrofusca* demonstrated by trapping in deciduous rain-forest at Kumasi, Gold Coast, in 1950.

Site	23/iv-14/v		14/v-4/vi		4/vi-25/vi		25/vi-16/vii		16/vii-6/viii		6/viii-27/viii		Total for 18 weeks		Ratio of <i>nigrofusca</i> to 100 <i>palpalis</i>
	<i>palpalis</i>	<i>nigro-fusca</i>	<i>palpalis</i>	<i>nigro-fusca</i>	<i>palpalis</i>	<i>nigro-fusca</i>	<i>palpalis</i>	<i>nigro-fusca</i>	<i>palpalis</i>	<i>nigro-fusca</i>	<i>palpalis</i>	<i>nigro-fusca</i>	<i>palpalis</i>	<i>nigro-fusca</i>	
1 Aggregate of 3 traps	4	0	5	0	3	0	4	0	9	0	8	1	33	1	3
2 "	11	0	4	0	6	0	1	0	10	0	23	0	55	0	—
3 "	47	2	45	1	82	0	61	5	54	2	137	0	426	10	2
4 Position a	77	13	55	11	79	7	22	8	36	3	67	32	336	74	22
" b	69	4	78	1	76	1	33	1	44	3	140	8	440	18	4
" c	87	9	81	6	69	8	105	1	20	3	23	0	385	27	7
5 Aggregate Position a	233	26	241	18	224	16	160	10	100	9	230	40	1161	119	65
" b	35	6	45	30	102	119	16	29	32	8	78	7	308	199	8
" c	35	3	10	1	7	3	10	1	5	0	21	0	88	8	9
6 Aggregate of 3 traps	52	5	14	7	5	1	7	0	7	3	9	0	94	16	17
7 "	122	14	69	38	114	123	33	30	44	11	108	7	490	223	—
	10	0	6	0	4	0	6	1	9	0	17	1	52	2	4
	12	0	2	0	4	1	4	0	3	0	22	0	47	1	2
Total	439	42	372	57	437	140	269	46	229	22	545	49	76%♀	50%♀	
<i>nigrofusca</i> /100 <i>palpalis</i>	10		15		32		17		10		9				

information could thus be obtained by one trap-boy instead of the two or more teams of fly-boys which would otherwise be required.

The survey, conducted throughout by the Scientific Assistant, Mr. Nowosielski-Slepowron, was started in May 1950, with 21 traps disposed around the periphery of the area, 5 to 15 yd. from the forest edge, in definite trapping sites marked with pegs. These traps were visited daily.

The point of outstanding interest in this experiment was the capture of *G. nigrofusca* as well as *G. palpalis* as soon as trapping started. The first-named species had not been caught here before by fly-boys. It appeared at certain trapping sites only, but it continued to be caught in these places during the subsequent period of the investigation. The catches for *G. palpalis* and *G. nigrofusca* for the first 18 weeks, during which trapping sites remained constant, are given in Table II.

The table is presented, not as a study on the biology of the forest tsetse, but to indicate the possibilities of making such a study by means of traps. When the total catches for the period are considered against topography (fig. 1), it can be seen that the traps give a clear indication of a disparate distribution of the two species of *Glossina* which bears a relationship to recognisable features in the terrain. Although *G. palpalis* appeared all around the periphery of the area, the catches in traps adjacent to tall forest were, on the average, 18 times greater than catches near the secondary bush. There was evidently a definite association of this tsetse with the heavier type of forest. The distribution of *G. nigrofusca* was more sharply demarcated. It appeared regularly at only two sites, nos. 4 and 5, at the western end of the College grounds, and here it was caught in some numbers. The section covered by these traps was adjacent to the densest and tallest parts of the surrounding forest, with the greatest density of tall trees interlaced with lianas and creepers. The ground here sloped steeply down to a valley bottom clothed throughout by the same type of dense, tall, impenetrable forest. This western edge of the College grounds, in fact, was the part which came closest to any of the several wooded valleys of the neighbourhood. Further indication that *G. nigrofusca* has a more restricted habitat than does *G. palpalis* comes from examination of the proportions between the two species shown in the final column of Table II, the former species being well represented only at sites 4 and 5.

A further point that can be seen from the table is that correct siting of the trap is of even greater importance for the capture of *G. nigrofusca* than for *G. palpalis*. At site 5, positions b and c (all marked by pegs and kept constant throughout the experiment) were within 8 and 25 yards, respectively, of position a, and the traps in both the former positions were easily visible from a, yet their catches, for each species of tsetse, never approached the high figures taken at position a. At site 4 there was no significant difference between the three positions for *G. palpalis*, but an appreciable difference for *G. nigrofusca*. It is interesting that at position 5a over half of the total catch of 199 examples of *G. nigrofusca* was taken within the short period of three weeks.

In a direct comparison between traps and fly-boys made for seven days in October 1951, in this area where *G. nigrofusca* was being taken, two teams, each of two fly-boys under a very competent Field Assistant, failed to catch a single example of this species although they took 158 of *G. palpalis*. During the same period, the traps captured 160 examples of *G. palpalis* and 7 of *G. nigrofusca*. Further comparison was obtained from simultaneous trapping and fly-boy rounds on the other side of Kumasi in a valley which was being cleared for the Gold Coast Regional College. In this area, boys and traps, working from July to October 1951, made the following catches:

	<i>G. palpalis</i>	<i>G. nigrofusca</i>	Ratio of <i>nigrofusca</i> to 100 <i>palpalis</i>
Fly-boys	506	2	0.4
Traps	293	10	3.4

The proportion of *G. nigrofusca* to *G. palpalis* in traps is similar to that found in the Prempreh College grounds at sites 1, 3, 6 and 7, which were near to, although not right in, a good habitat of *G. nigrofusca*. On the other hand, the proportion between the species shown by fly-boys working in the same place is something quite outside this range of values. It is evident that hand-catching falls very far short of showing the true numbers of *G. nigrofusca* which are present.

A search, using Nash's resting-haunt technique (Nash & Davey, 1950), was also made by Nowosielski-Slepowron accompanied by skilled fly-boys in the forest close to the places where *G. nigrofusca* was being regularly caught in the Prempreh College grounds. Only one tsetse of the *fusca* group was seen and this was not caught. The Ashanti rain-forest is dense and impenetrable, a mass of lianas and creepers impede one's movement and vision, and the resting-haunt technique in searching was found extremely difficult to apply.

In further trapping work by the same observer, *G. pallicera* was caught in small numbers in the high forest around Kumasi.

To summarise, these trials demonstrated that, under forest conditions, 'Morris' traps, requiring a minimum of trouble and personnel, are effective in taking *G. palpalis* and *G. pallicera*, and the very elusive *G. nigrofusca* when fly-boys have either failed to detect their presence or have given a quite unrepresentative picture. It is reasonable to conclude that traps in the forest, properly employed, can provide a sensitive means of sampling in which the numbers of flies caught bear a relation to habitat, behaviour and movement.

Sampling G. longipalpis in coastal thicket.

A further problem arose in a part of the coastal belt with degraded, secondary forest, in connection with the formation of a leper settlement at Ankaful, 6 miles north-west of Cape Coast. This undulating country is dominated by dense, monotonous thicket, broken only by farms and occasional open glades along drainage lines. The vegetation is essentially that described at Takoradi (Morris, 1934, p. 310). At least three species of *Glossina* were known to be present: *G. longipalpis* was widespread throughout the thicket, being the most common; *G. palpalis* occurred more particularly along rivers or in evergreen clumps on marshy land; and *G. medicorum* was recorded occasionally over most of the coastal area.

The leper settlement lay in a clearing, a mile long by three-quarters of a mile wide at its broadest, narrowing to 300 yd. at one end, on flat ground flanked by a small hill on the northern side on which were staff houses. Surrounding the clearing on all sides was the remarkably uniform thicket, composed of an impenetrable mass of straggling stems of regenerating trees, 7 to 10 ft. in height with practically no tall trees. Creepers and lianas were few, yet the density was so great that it made it almost impossible even to creep through and reduced visibility to a matter of 5 to 10 yd. The clearing contained farms and houses in which a colony of lepers was being settled. The colony was eventually to be self-supporting, producing food crops, fruit and livestock.

Ankaful presented a problem, mainly of animal trypanosomiasis, which occurred all along the coastal belt, where the presence of tsetse, *G. longipalpis* in particular, made it a virtual impossibility to rear livestock for the local markets. The big demand for meat had to be met by importing cattle and sheep by sea from Nigeria or on the hoof from 500 to 800 miles to the north. Serious losses from trypanosomiasis had occurred in farms lying just within the fly-belt of *G. longipalpis*, only 15 miles from Accra. Consequently, it was decided to make a thorough survey at Ankaful, coupled with a long-term study on control measures. Although it was known that fly-boys could be used for reliable comparative work on *G. longipalpis*, the relationship of such samples to true incidence was not known. For this reason

and because of practical difficulties similar to those around Kumasi, it was decided to see if traps could be utilised for the Ankaful investigation also. Experiments started at the end of July 1951, under the charge of Mr. Nowosielski-Slepowron.

As soon as the traps were put out, *G. longipalpis* was caught. *G. palpalis* was also taken, but in much smaller numbers because of the nature of the country. At the beginning of the investigation, eight traps were placed around the settlement area under the charge of one trap-boy, who cleared them daily. During the first eight weeks, 109 examples of *G. palpalis* (54% ♀) and 417 of *G. longipalpis* (53% ♀) were taken, giving a ratio of 383 of *longipalpis* per 100 of *palpalis*. There was no doubt that the traps would prove an adequate instrument for sampling *G. longipalpis*.

On 8th October, eight more traps were put out, nearer to the edge of the bush than the original traps, which stood well within the clearing and close to the settlement buildings. The traps can be considered in three distinct groups in relation to the areas which they were sampling. Group 1 consisted of the eight original traps, standing among farms or close to occupied buildings, well within the clearing. Group 2 consisted of four of the new traps placed close to the thicket edge along the northern side of the settlement, which is skirted by a motor road with the main settlement entrance and staff buildings on its farther side. Group 3 had four traps placed along the southern edge of the clearing, where unbroken and undisturbed thicket extended for 2 to 3 miles southward and for much greater distances to the east and west. There were no material changes in these conditions between 8th October and 19th November 1951, and a study of the catches during these six weeks is significant. Comparisons are simplified by considering only four traps in group 1, taking the catches from alternate traps. The results are shown in Table III.

TABLE III.

The influence of environmental disturbance on the incidence of *Glossina longipalpis* as shown by trapping at Ankaful Leper Settlement, near Cape Coast, Gold Coast. Total catches of tsetse flies for 6 weeks, 8th Oct.–19th Nov. 1951.

Area	<i>G. palpalis</i>			<i>G. longipalpis</i>			Ratio of <i>longipalpis</i> to <i>palpalis</i>
	♂	♀	Total	♂	♀	Total	
1. Four traps in original sites in clearing close to settlement; continual disturbance	5	2	7	20	31	51	7.3 : 1
2. Four traps at edge of clearing on higher ground adjacent to road and staff houses; frequent disturbance	7	7	14	40	99	139	9.9 : 1
3. Four traps at edge of clearing remote from road, on lower ground sloping towards marsh; no disturbance	10	15	25	261	393	654	26.2 : 1

The significance of this table lies in the nice gradation shown by the numbers and ratio of *G. longipalpis* in relation to the amount of disturbance of the area sampled (the areas are given by the same numbers as the trap groups sampling them). Area 1 was constantly disturbed by the presence of people on the farms and around the buildings. Flies entering these traps had to cross from 15 to 50 yd. of open clearing. In area 2 there was less immediate proximity to people, but disturbance did occur through the entrance along the motor road traversing the bush just beyond the clearing edge and around the bungalows a hundred yards to the north. In area 3, showing nearly five times the catch of *G. longipalpis* in

area 2 and 12 times that in area 1, the bush was completely undisturbed. The studies of this species at Takoradi had shown that this fly was almost eliminated in one area when it was subjected to disturbance by farming and hunting (Morris, 1934). It is hardly necessary to emphasise the contribution which findings of this nature could make when control is considered.

Comparison of the relative efficiencies of traps and fly-boys was made by working two teams, each of two boys under a Field Assistant, for 13 days in the area being trapped. In the first team the boys were expert in catching *G. morsitans* as well as the more easily taken *G. palpalis*. The second team had experience with *G. palpalis* only. In the 13 days, 16 traps took 370 examples of *G. longipalpis*, the first team (*morsitans* experts) took 108, and the second team failed to get a single specimen although working the same ground and catching *G. palpalis* quite well. The *morsitans* boys improved their rate of catching appreciably during the trial. They found that *G. longipalpis* very rarely followed them, only three specimens being caught off themselves. As in the Takoradi investigations (Morris, 1934) and as Nash & Davey (1950) later found in Nigeria, most of the flies were caught off the vegetation, where they were observed resting on leaves or tree stems. They were often seen warming themselves on the top of a leaf in the sun. Although the flies did not follow the boys while moving they would appear around them when they stopped and then settle nearby. The boys soon devised the technique of disturbing the bush with a stick as they walked along and stopping as soon as an example of *G. longipalpis* was seen or heard in order to watch for it to settle and afford the opportunity for its capture. On one occasion four flies were caught settling on the same fallen branch in a matter of minutes. The catches made during this trial are given in Table IV.

TABLE IV.

The relative efficiency of traps and fly-boys in sampling *Glossina longipalpis* in coastal savannah thicket near Cape Coast, Gold Coast. Total catches of tsetse flies for 13 days in October 1951.

	<i>G. palpalis</i>		<i>G. longipalpis</i>		Ratio of <i>longipalpis</i> to <i>palpalis</i>
	Total	% ♀	Total	% ♀	
16 traps	36	61	370	56	10.3 : 1
2 fly-boys experienced in catching <i>G. morsitans</i>	64	27	108	7	1.7 : 1
2 fly-boys, no experience with <i>G.</i> <i>morsitans</i>	66	21	0	—	

Apart from the greater number of *G. longipalpis* caught by traps, two important features emerge: first, the much higher ratio of this species to *G. palpalis* appearing in traps; and second, the marked difference between sex ratios shown in catches by traps and by boys. Throughout the investigation, the traps took males and females of *G. longipalpis* in almost equal numbers. The boys' catches, on the other hand, showed the low female percentage which is characteristic of *G. morsitans* taken by moving teams in its habitat in the inland savannah. It was quite evident that the traps were giving a more truly representative sample of the population of *G. longipalpis* present.

Another important characteristic of trapping is that the catches did not exhibit the big day-to-day variability so noticeable in catches by fly-boys, as can be seen from the series of daily records for *G. longipalpis* shown in fig. 2.

A suggested explanation is that the boys, working on the fly-round method, were not always at places of maximum concentration of the flies at the times of their greatest activity, which varies markedly during the day, whereas the traps were in position all the time and consequently at whatever time periods of activity took place. Whatever the explanation might be, the much steadier rate of catching by traps would suggest that greater reliance could be placed on this method of sampling rather than on that by fly-boys.

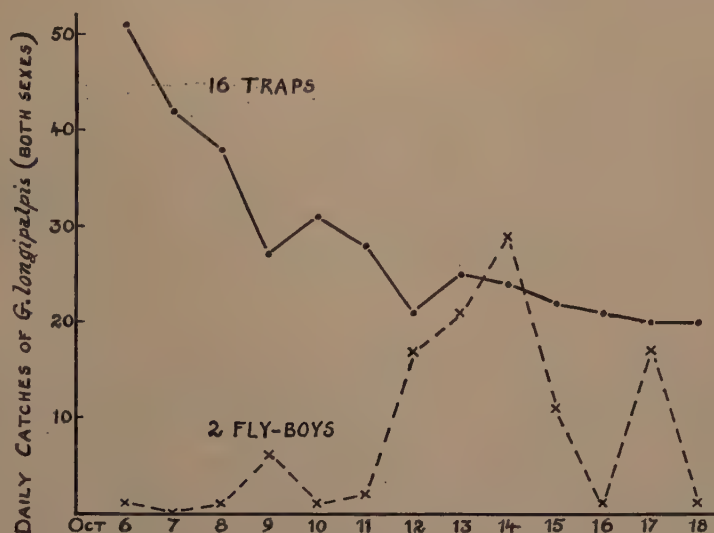


Fig. 2.—Comparison of traps with fly-boys for sampling *Glossina longipalpis* in coastal thicket, Cape Coast, Gold Coast, 1951.

The figure shows another interesting feature: a 50 per cent. drop in the numbers of flies caught during the first week, after which the catching tended to level off to a more constant rate. This catching-out effect was observed in the case of *G. palpalis* and of *G. tachinoides* when trapping in their feeding grounds in the inland savannah (Morris & Morris, 1949) and in the case of *G. pallidipes* Aust. in Uganda (Morris, 1960). It is explained as a skimming off of a part of the local tsetse-fly population, the hungry and therefore more trappable flies, which concentrate in the neighbourhood of food-hosts. The more uniform rate of catching, which follows the sharp initial fall, represents the steady flow of hungry flies into the feeding ground from the neighbouring extensive tsetse community.

Glossina medicorum is known to be present in the transitional type of forest vegetation along the coast. Ankaful did not appear to offer a very favourable habitat, but the traps here took four specimens (3♂, 1♀), and the expert fly-boy team caught two males. At Pokoase Farm near Accra, 78 miles to the east and still within the coastal belt, 'animal' traps were found equally effective in sampling *G. longipalpis* and *G. medicorum* as well as *G. palpalis*.

The study of *G. longipalpis* is one of the most urgent problems in West Africa at the moment, not only because of the extensive areas of potential grazing and farm land which it occupies, but because so little is as yet known of its ecology and control. Even the matter of surveying this species presents practical difficulties not yet overcome, as the fly-boy and trap comparisons made amply clear. The weakness of survey is not surprising considering that the development of

methods cannot progress without a knowledge of the insect's biology. The Ankaful experiments show that traps not only prove a more economical and efficient means of sampling *G. longipalpis*, but that they give a closer approximation of what must be the true incidence of this fly than do the most skilled fly-boys.

The uses and limitations of trapping.

The evidence from the foregoing experiments and from those conducted elsewhere, shows that traps can prove a valuable means for sampling a number of species of *Glossina*. It is instructive to discuss the ways in which traps are superior to human observers in this respect.

Standardisation of data.

This is perhaps the most valuable quality of all, particularly in the more fundamental types of research in which large numbers of observations are required, often from widely separated localities, yet with strict comparability of data, and perhaps with the need for replicates conducted under, as nearly as possible, identical conditions. The fact that traps are largely mechanical, with the observer variable reduced to a minimum, gives them their great value for this type of biological work in which so many uncontrollable variables already exist.

Reliability.

Slightly different in concept from standardisation, which concerns the statistical value of the data collected, is the question of the greater reliance which can be placed on a mechanical means of sampling. Mechanisation eliminates factors such as fatigue, sickness, inclement weather, or a local market, dance or funeral, which can interfere seriously with the smooth functioning of a routine dependent entirely on the human observer. The trap-boy, indeed, is liable to these disturbances, but to a lesser extent, since he is a much more carefully selected and trained individual and on a higher staff grade than is the fly-boy. But, should he be unable to carry out his rounds, the trapping continues uninterrupted and the samples can eventually be taken and an allowance calculated for the one or two days of interruption (Morris, in press). In very important trap rounds, the writer places two boys in charge to ensure continuity. A further aspect of the value of reliability lies in the fact that, since traps are fixed in position and work at a constant rate, any irregularity from a fairly smooth trend can only be due to significant changes in the behaviour or environment of the flies and will serve as indicators of this, as the Gold Coast experiences well show.

Economy of staff.

It has been found in practice that a single trap-boy can easily operate 20 or more traps on daily visits or double that number by visiting on alternate days. To assess the greater economy of trapping, consider the case of one trap-boy operating 20 traps sampling five miles of riverine fly-belt. The equivalent amount of observation by fly-boys would require three teams, each consisting of two boys and a Recorder—nine staff members in all. The same trap-boy, making alternate day visits to 40 traps, would be doing the work of 15 to 18 people; and the information obtained from traps would be of a higher order of accuracy than that from fly-boys. This hinges on the next consideration.

Greater efficiency in sampling.

Working with *G. palpalis* and *G. tachinoides* in the Gold Coast, Morris & Morris (1949) showed that, in the dry season, traps can catch as many as ten times the number of flies as can fly-boys, thus providing unquestionable evidence of the

existence of a much greater fly population during this period than would appear from catches by boys. A similar experience was found with *G. palpalis* in the rain-forest of Liberia (Morris, in press). In the cases of *G. nigrofusca* and *G. longipalpis* described above, trap catches showed a numerical superiority and also a percentage of females for both species (50 and 56%, respectively), which was much closer to the expected sex ratios in natural tsetse populations than were the figures shown by boys, indicating that traps take more representative samples of the population than do boys. A similar phenomenon was found with *G. pallidipes* in Uganda (Morris, 1960) with 75 per cent. females in trap catches against 10 per cent. by boys. The greater number of females of *G. longipalpis* and *G. pallidipes* caught by traps was sufficient to explain the gross numerical superiority of this means of sampling.

The main reason for the greater catching capacity of traps probably lies in the fact that they are in position every day for 24 hours a day, thus being at hand to tap the periods of activity of the flies whenever these occur. Boys, with their limited hours of catching, may strike this activity period on one day and miss it completely on another, especially when weather conditions—wet, cold, or cloudy days and the winter harmattan wind—damp it down to a few hours a day. When it is kept in mind that the activity period in female flies is even more restricted than in males, it can be seen that the same reason would account for traps taking a greater proportion of females in the game-feeding species, *G. longipalpis*, *G. pallidipes* and *G. nigrofusca*, which are not attracted by man and investigate possible hosts only when hungry. This supposes the efficiency of traps to lie in their resembling a natural host and attracting feeding tsetse, the theory which was behind the original design of the 'animal' trap, in support of which Morris & Morris (1949) produced evidence from the Gold Coast. The whole question of the attractive powers of traps is not yet fully understood.

In the Gold Coast studies quoted above, the marked dry-season superiority of traps was reversed during the rains, trap and boy catches being approximately equal during June and July, while from August to October boys took more flies than did traps in the proportion of 4:1. The explanation given was that the dry-season range, a narrow, easily trappable belt along the waterside, is extended during the rains by the greatly increased humidity and leaf cover. Traps then lose the advantage of being constantly within the habitat of the flies, whereas the boys are free to move about and can locate the flies in their temporary resting places, which may be well away from the primary dry-season habitat. Visibility is also lower, to the disadvantage of the traps; and the regular supply of hosts at the river bank, so important a factor in good trapping sites, is lessened by the availability of many other sources of water. One is left with the conclusion that, although the catches made by traps working under ideal conditions approximate more closely than do fly-boy catches to the true numbers of tsetse present, yet under less favourable conditions both methods probably give inadequate samples. The question is raised of how the performance of traps could be improved.

Increased efficiency in trapping might be attained in three ways:

1. By increasing the number of traps in each locality, thus overcoming the limited range of attraction of this type of unbaited trap, which may not extend beyond five to ten yd. In attempts to trap-out the populations of *G. palpalis* and *G. tachinoides* from sacred groves in the Gold Coast in 1942-44 (Morris & Morris, 1949), it was shown that trebling the number of traps increased the total catches three times, i.e., a much closer representation was obtained of the populations present. By that time the two groves, each approximately an acre in extent, contained 22 and 20 traps each and it was difficult to find new sites more than five or six yards from existing traps; it was hard to imagine any tsetse not

encountering one or more traps during the day. A steep reduction in the catches of flies during the next six months left no doubt that the traps were rapidly and substantially reducing the tsetse populations, which means that over this period their catches came very close to the true number of adult tsetse present in the two groves. The fact remains that, however much trouble may be involved in producing a catching-out effect such as the above, this is the most conclusive answer to the question of population counts and is of far greater value than estimates involving complicated statistics based on samples often of questionable reliability.

2. Trapping efficiency could undoubtedly be increased by added knowledge of the habits of the species being trapped, a matter which received close study recently in Liberia. The result, however, might be a too specialised trap, especially if actuated by scent or bait, highly successful for some species, much less so for others, thereby losing the advantages of simplicity and standardisation. In population studies a uniform rate of sampling, even involving smaller numbers, may give more accurate long-term or over-all pictures than do methods taking much higher numbers in some instances and lower in others.
3. There could be intrinsic improvements in the traps. It has been shown that a proportion of the tsetse entering the catching cage eventually escape (Morris, in press). The magnitude of this escape rate with *G. palpalis* and *G. tachinoides* in the north of the Gold Coast was shown by M. G. Morris (1950), who demonstrated a 43 per cent. superiority in the catches of these species in a trap covered with hessian impregnated with DDT. The increased catch could only be explained by the delayed paralysing effect of the insecticide on the flies preventing their escape from the cage later. The improved performance of the treated trap, however, was not uniform, being subject to a gradual loss of toxicity in the hessian and to a wide seasonal variation, with failure of toxicity under wet and cool conditions in the heavy rains but recovery with the succeeding hot, dry weather. However desirable a 40 per cent. increase in the efficiency of a trap may be, it is unwise to achieve this at the cost of one of its most valuable assets, that of standardisation. Under special circumstances, an increased efficiency obtained by the addition of an insecticide might be useful; for example, in the use of traps for protective purposes in places of high man-fly contact, or for producing a catching-out effect rapidly. The scientific value of the trap would be diminished, however, because of an unmeasured death-rate of flies which have walked over the trap and received a fatal dose of insecticide but not subsequently entered the cage. The trap sample, then, would be incomplete as well as subject to irregularity. It is evident that other methods of improving the performance must be sought, and this is still the subject of close attention. At present the escape rate can only be overcome by more frequent visits, or by saturating the area with traps so that flies escaping from one may be caught and held by another.

To sum up, it is evident that, for certain species of tsetse, traps provide a much more efficient means of sampling than do fly-boys, the superiority being both practical, in cheapness and ease of manipulation, and basic, in that the fly population is being more correctly sampled. Indeed, the only conclusive means of arriving at a true population count, by catching out all adult tsetse present, can be achieved or very closely approximated by the use of traps.

There remains the question of what could be the rôle of traps in survey. In the first place, it has been pointed out that a survey cannot be more than superficial

without a knowledge of at least certain points in the biology of the animal concerned. In acquiring this knowledge, traps have a very valuable rôle, particularly in the case of the more elusive species of tsetse. When it comes to extending these studies to the survey of wider areas, the apparent obstacle of the immobility of traps compared with boys becomes less real when the fallacies of too rapid a survey are borne in mind. It has been shown that a minimum of four days' observation in each locality is necessary for even a 90 per cent. reliability in the sample. We also know that traps show at once the presence of many species of tsetse which are not easily detected by fly-boys, and that over a period, whether of four days or four months, trap catches give a more reliable index of the numbers present. The use of traps as a survey instrument in place of boys, then, appears practicable, and the possibility of doing so has recently been demonstrated in Liberia (Morris, in press). A folding trap was developed to facilitate transport, and on navigable rivers a trap mounted in the bow of a canoe made astonishing catches of *G. palpalis*.

It might be thought that these studies are highly specialised and concern only one small genus of insect peculiar to tropical Africa. On the contrary, this actual type of trap, unbaited and unmodified, is regularly taking insects of the following families of Diptera—TABANIDAE (a number of species), CULICIDAE and MUSCIDAE—and of the orders Lepidoptera, Hymenoptera, Rhynchota and Coleoptera. Of wider interest are the more fundamental lessons which have been gained on trapping. Mammalogists and entomologists in many regions have developed trapping as a valuable method of studying populations, mainly with the secretive small mammals, and with nocturnal insects by means of light-traps. The present work has demonstrated the technical and practical values of a simple and inexpensive trap, independent of an electric supply and capable of use in numbers almost anywhere, for studying the diurnal insects also. The advantages of a simple mechanical means of sampling are especially pronounced in primitive and underdeveloped countries, where so many problems of a biological nature exist, but where the building up of a reliable observer personnel is often a matter of the greatest difficulty.

Summary.

A detailed tsetse-fly survey of 41,000 sq. miles in the inland savannah zone of the Gold Coast (Ghana) was completed in five years using an entirely African staff and working only six months each year.

This undertaking was made possible because studies of the species of *Glossina* concerned, *G. palpalis* (R.-D.), *G. tachinoides* Westw. and *G. morsitans submorsitans* Newst., had provided knowledge of their habitat, food-hosts and activity rhythm, on which could be based a standard yet simple survey technique, within the compass of independent native teams.

The restricted activity rhythm of the flies, subject to both daily and seasonal variations, meant that nothing less than a 'day' of eight hours of observation could be accepted as a valid sampling unit.

With tsetse flies at low densities, a day's search was inadequate, giving only 40 per cent. reliability compared with 6-day observations. Four days' search gave 90 per cent. reliability and was adopted as the minimum period of search.

Each survey team was under the charge of a Recorder or Field Assistant, with fly-boys devoting the whole of their time to catching. Comparability of survey data was ensured by keeping teams to the same size, two fly-boys being the optimum, since it was found that the size of the catch was not always proportionate to the number of boys, the relationship varying according to a variety of factors.

In surveying *G. palpalis* and *G. tachinoides* the 'picket' system of more or less stationary teams, measuring their catches by the 'day', was found overwhelmingly superior to the 'fly-round' technique.

The survey procedure described for savannah woodland country was not effective in sampling *G. nigrofusca* Newst. in the Ashanti forest or *G. longipalpis* Wied. in the coastal savannah, but Morris's 'animal' traps were found to take both species readily in places where they had not been detected or only occasionally caught by fly-boys. Experiments showed that trap catches were superior, both numerically and in giving a more representative sample of the tsetse population present. It was also shown that trapping could be a valuable means of studying the biology of these two 'man-shy' species of *Glossina*. Traps were also effective in taking *G. pallicera* Big. and *G. medicorum* Aust.

The greater value of traps compared with fly-boys as a means of sampling various species of tsetse fly is discussed, and suggestions are put forward for developing trapping as a method of survey.

The type of trap employed is regularly catching insects of several other families, including a number of species among the TABANIDAE, and of several other orders.

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STUDIES ON THE LIFE-HISTORY OF *AÊDES* (*SKUSEA*) *PEMBAENSIS* (THEOBALD) (DIPTERA, CULICIDAE).*

By C. BROOKE WORTH,† JACINTO DE SOUSA †
and M. PAUL WEINBREN §

The isolation of viruses from *Aedes* (*Skusea*) *pembaensis* (Theo.) in 1959 (Kokernot, R. H., McIntosh, B. M., Worth, C. B. & Morais, A. T., paper in preparation on Lumbo virus) and 1960 (Arthropod-borne Virus Research Unit, Johannesburg, unpublished information) brought new medical importance to this mosquito. It was already known as a man-biter that freely enters houses (Aders, 1917), while it had also been shown to be a vector of filariasis in Kenya (Heisch, Goiny & Ikata, 1956).

Knowledge of the life-history of *A. pembaensis* seems confined to the facts that the chief larval habitats are crab holes in saline environments (Aders, *op. cit.*) and that the females lay their eggs on crabs (Goiny, van Someren & Heisch, 1957). Reported distribution of the species includes sea coasts and coastal islands of Kenya and Tanganyika (Edwards, 1941), Madagascar (Edwards, *op. cit.*) and Mozambique as far south as Inhaca Island (Pereira, 1958).

The viruses mentioned above were isolated from adults of *A. pembaensis* collected during biological and other studies made on this species during March in 1959 and 1960 at Lumbo on the coast of northern Mozambique. The information gained in these studies is presented here.

Materials and methods.

Mosquitos were collected individually, in cotton-stoppered glass tubes measuring 15 x 90 mm., by locally recruited boys. In 1959, the boys were instructed to catch mosquitos as they came to bite; hence very few males were taken. In 1960, however, the procedure adopted was to catch not only biting individuals but also those resting on vegetation, on the ground, in crab holes, etc., so that significant numbers of males as well as females were collected. Identifications were made with a 5x hand lens while the mosquitos were still in the tubes. For the purposes of the virus study, all species of Culicine mosquitos were included.

During the first expedition to Lumbo, a number of habitats was sampled; these included grassy slopes at the margin of the bay separating Lumbo from the Island of Mozambique, a tidal estuary leading from the bay, and several sites extending inland along a road as far as seven miles from the head of the estuary. On the basis of mosquitos captured and viruses isolated from them at that time, the 1960 collections were limited to the mangrove-bordered tidal estuary and a small patch of trees and bush one mile inland along the road.

Mosquito catches were roughly uniform in size throughout both years of study. About a dozen boys were employed and they were given between 800 and 1,000 empty tubes each morning. Thus, although the time required to get a mosquito into each tube varied with the number of boys reporting for duty, the weather, etc., the numbers of mosquitos eventually obtained each day were relatively

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† The Rockefeller Foundation.

‡ Instituto de Investigacao Medica, Lourenco Marques.

§ South African Institute for Medical Research.

constant. It is therefore possible to compare daily fluctuations in the species composition of the mosquito populations with a fair degree of assurance.

Blood from engorged mosquitos was smeared on filter papers for identification of its source. At the Johannesburg laboratory, precipitin tests were performed by a modification of Weitz's (1956) method.

Crabs inhabiting the estuary were also collected for identification. The only species found bearing eggs of *A. pembaensis* was identified, through the courtesy of Mr. V. G. James, at the University of the Witwatersrand, Johannesburg, as *Sesarma meinerti*, the same species as was chiefly implicated in Kenya (Goiny, van Someren & Heisch, *op. cit.*). Mosquitos were not reared from these eggs, but eggs laid by gravid females of *A. pembaensis* on filter paper displayed identical morphology.

Results.

Breeding site.

The presence of eggs of *A. pembaensis* on *S. meinerti* was readily confirmed. These were arranged in horizontal rows principally on the first or proximal large segment of the front legs, on both the medial and lateral surfaces. Populations of this crab were found along the bay shore as well as at several sites bordering the more sheltered estuary, but crabs bearing mosquito eggs were restricted to the inner reaches of the estuary which became flooded only during the higher tides.

Crab burrows were usually situated among mangrove roots or in their immediate vicinity. Only a few were found in open situations and these were never on the sand-and-mud flats but rather in fringing embankments or dunes. The ordinary burrow was tortuous, with radiating sub-surface branches, making it difficult to dig out the crabs. The average diameter of a burrow was between one and two in. and the maximum depth was about two ft. Most burrows were examined at low tide, when only some of them contained standing water although all were moist. No intensive search for mosquito larvae was made, and none was seen by casual observation.

Behaviour at burrows.

When a crab entered or left its burrow during the day, mosquitos, which proved to be *A. pembaensis*, could be seen to fly out momentarily. The presence of a human being was then sometimes a stimulus causing a mosquito to bite in bright sunlight, but ordinarily the disturbed insects flew back into the burrow almost at once. Here they could be seen near the entrance, engaging in a number of rhythmic darting sallies before they settled down. The manoeuvre resembled some sort of mating dance. All attempts to observe swarms of males both in day-time and as it grew dark failed.

TABLE I.

Numbers of *A. pembaensis* and other mosquitos collected at Lumbo in 1959 and 1960.

Year	<i>A. pembaensis</i>		Other mosquitos	Percentage of <i>A. pembaensis</i>
	Males	Females		
1959	—	9856	6385	60.7
1960	2993	9404	5682	68.6

Populations and dispersal.

The numbers of *A. pembraensis* taken in 1959 and 1960 by the methods described are compared in Table I with the total numbers of other mosquitos caught at the same times. It will be seen that *A. pembraensis* was the predominating species on both occasions. The only other mosquito that even approached it in relative abundance was *A. (Aëdimorphus) albocephalus* (Theo.), of which 2,180 females were taken in 1959 and 3,348 individuals of both sexes in 1960. Some 25 additional species of mosquito were captured in lots totalling less than a thousand specimens of each.

Females of *A. pembraensis* ranged inland freely to a distance of one mile from the estuary but penetrated in smaller numbers as far as four miles inland (Table II).

TABLE II.

Numbers of *A. pembraensis* in average catches at various collecting sites.

	Distances inland from estuary breeding area		
	A. Females		
	0 mi.	1 mi.	4 mi.
1959	646	582	177
1960	478	537	—
	B. Males		
	0 mi.	1 mi.	—
1960	364	3	—

The data for 1960, showing females to be apparently more abundant one mile inland than at the estuary itself, are misleading because of the fact that in a given daily catch at the estuary many of the allotted tubes were occupied by males. As regards males, it is strikingly shown that only an insignificant number of them wandered inland.

Feeding: Reactions to bites.

Females of *A. pembraensis* in tubes, brought in from the field, were given opportunities to bite three Caucasian adult males. Two of these individuals were apparently unpalatable, for most mosquitos refused to bite them. The bites that were sustained were painless and no visible cutaneous reaction followed. The third individual proved to be highly attractive to most of the mosquitos. He complained of discomfort while being bitten, and the sites subsequently became elevated and red, itching with decreasing severity for ten days.

Precipitin tests.

Sixty-four blood specimens from engorged females of *A. pembraensis* collected in 1959 at various sites at Lumbo all gave positive tests with anti-human serum. Of 25 blood specimens from *A. albocephalus* collected at the same sites, 24 were positive with anti-human serum, the remaining one with anti-goat serum. Blood was not collected from other species.

Virus isolations.

Eight strains of an apparently new virus were isolated from females of *A. pembraensis* in 1959. In 1960, four strains of the same virus were obtained, also

from females of *A. pembaensis*, and in addition two strains of a virus of the Bunyamwera group were isolated from females of this species. Human sera collected from residents of Lumbo in 1959 showed only a low incidence of antibodies against the virus isolated in that year. No viruses were obtained from *A. albocephalus*. Of thirty sera collected in 1960, at least four showed neutralising antibodies against the first virus and six had haemagglutinating antibodies against Bunyamwera virus.

Discussion.

The apparent predilection of *A. pembaensis* for *S. meinerti* as an egg-bearing vehicle places a biological restriction on the range of the mosquito. Since the crab itself is confined to tidal areas, and not even all crab colonies are utilised by *A. pembaensis*, the medical importance of this insect must be limited to the flight range of the female from its breeding area. Thus, in a given locality, *A. pembaensis* may have an intense bearing on public health, while a few miles distant its effect will be *nil*. It is to be assumed that however far a female may fly, it must ordinarily make the return trip to a crab colony to oviposit. The anomalous finding of larvae of *A. pembaensis* in pineapple axils (Teesdale, 1941) suggests that occasionally a gravid female behaves otherwise, but whether this contributes successfully to the maintenance of the species is unknown.

The negligible numbers of males of *A. pembaensis* caught at the inland site indicates that this sex is relatively sedentary. Since there is probably no need for males to wander in search of food, their lack of movement favours survival, for the open reaches of the estuary beyond the sheltering mangroves are often windswept. Failure to observe swarms of males, in addition to the suspected mating dance seen within the entrances to crab burrows, suggests that the species may be stenogamic in this regard. Attempts to establish self-perpetuating insectary colonies might therefore not be faced with difficulties in securing mating.

Populations of *A. pembaensis* in 1959 and 1960 were roughly equivalent, as were also the number of viral isolates secured from this species. However, the residents of Lumbo stated that 1959 had been a year of normal rainfall, while 1960 was exceptionally dry. The effects of such climatic variation on mosquitos breeding in tree holes or standing fresh-water pools may be seen in the size of catches of five species of *Stegomyia* (707 in 1959; 397 in 1960) and of *Culex* (*Culex*) *univittatus* Theo. (352 in 1959; 4 in 1960). It is thus clear that the estuarine environment, depending on the orderly movement of the tides, is a far more stable one than the wholly terrestrial milieu with its variable rainfall history. Consequently the year-to-year cycles of viruses, filariae or other pathogens may be less precarious in *A. pembaensis* than in mosquitos bound to dry land.

The rôle of *A. pembaensis* as a virus vector remains controversial, even in the absence of transmission experiments in the laboratory. The evidence that it feeds exclusively on man may be biased in view of the fact that, in 1959, mosquitos were caught chiefly as they came to bite the mosquito boys. On the other hand this was true also for *A. albocephalus*, which likewise was shown to contain human blood in 24 of 25 instances but from which no isolations of virus were made in either year. The low incidence of antibodies to the 1959 virus in human sera, coupled with a rather high incidence of virus in *A. pembaensis*, suggested that the mosquito might acquire the virus from a non-human source. This conjecture could not be correlated with the evidence that human beings were the chief, or only, source of its blood-meals at Lumbo. On the other hand, Heisch (personal communication) has recovered filarial worms pertaining to several quadrupeds from *A. pembaensis* in Kenya.

The hypothesis that viruses might be acquired in the larval state was tested in 1960 by the collection of male mosquitos and *Sesarma meinerti* for attempted virus isolation. The results were negative, although perhaps an insufficient number of

specimens was processed (2,993 males of *A. pembaensis* and 105 examples of *S. meinerti*). Further evidence against this hypothesis is the fact that of the six lots of *A. pembaensis* yielding virus isolates at Lumbo in 1960, five were collected at the inland station, presumably the more important feeding area, and only one among the crab colonies.

Summary.

The observation in Kenya that *Aedes (Skusea) pembaensis* (Theo.) associates with the crab, *Sesarma meinerti*, was duplicated in Lumbo, on the northern coast of Mozambique. Eggs were seen abundantly on crabs inhabiting certain colonies among mangroves at the edge of a tidal estuary. Females of *A. pembaensis* were caught in gradually decreasing abundance at collecting sites progressively further inland from the head of the estuary, while males were almost totally confined to the breeding area. Swarms of males were not seen, and it was suspected that mating took place within crab burrows.

Populations of *A. pembaensis* in 1959 and 1960 were approximately equivalent at Lumbo, whereas some other mosquito species with fresh-water larval stages were reduced in 1960 owing to failure of the rains. The estuarine environment, regulated by the tides, is apparently more stable than are terrestrial habitats. Cycles of *A. pembaensis* may therefore be less variable than among dry-land forms.

Females of *A. pembaensis* disturbed at a crab colony were found to bite spontaneously in the day-time. Captured specimens showed a marked discrimination in biting preference for one human out of three tested. The preferred individual experienced a severe reaction to the bites, while the other two had none whatsoever.

Precipitin tests indicated human beings as the only source of blood in *A. pembaensis*, although the method of collection probably lends bias to this observation. Eight strains of an apparently new virus were isolated from females of *A. pembaensis* in 1959 and four in 1960. In addition, two strains of a virus of the Bunyamwera group were isolated in 1960. The source of these viruses and their significance in *A. pembaensis* require further investigation.

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THE MATING BEHAVIOUR OF *PIERIS BRASSICAE* (L.) IN A LABORATORY CULTURE.

By W. A. L. DAVID and B. O. C. GARDINER

*Agricultural Research Council Unit of Insect Physiology,
Cambridge.*

The culture of *Pieris brassicae* (L.), the large white butterfly, which is the subject of this paper, was originally established for work on systemic insecticides. It was started from eggs collected in the field in the late summer of 1950 and fresh stock has not been added since. No difficulty has been experienced in this laboratory in maintaining a large culture at all times of the year. In other laboratories, the same success has often not been attained, and reports of cultures dying out led the authors to determine the influence of certain factors on the health and productivity of the culture.

While carrying out this investigation, observations on other aspects of the biology of *P. brassicae* in captivity have been made, and these results are included in the present paper.

Equipment and methods.

The equipment and methods used in maintaining the culture in the normal way have been described and illustrated in two earlier papers (David & Gardiner, 1952; David, 1957). Much of the present work has involved modifying this procedure, and the changes involved are described in conjunction with each experiment.

The tests were conducted in the glasshouse in which the culture was maintained or in constant-temperature rooms in the laboratory. These rooms were maintained at 25°C. and 55 to 60 per cent. relative humidity. However, in strong light, the temperature in the test cage sometimes rose above that in the surrounding room and the humidity fell correspondingly. The figures quoted in connection with the experiments are those determined within the test cages.

A direct reading photometer, with a selenium photo-voltaic cell, made by Everett Edcumbe & Co. Ltd., was used to measure the illumination. According to the manufacturers, the colour sensitivity of the cell follows closely that of the human eye.

General observations on the adult insects.

Appearance.

During the ten years over which the culture has been established, adult insects have been produced at an estimated average rate of 200–400 per week or approximately 15,000 per annum.

About 95 per cent. of the healthy pupae gave rise to normal adults. Some distortion of the wings accounts for most of the abnormal 5 per cent., but a few recognised variants have been noticed and also two mixed gynandromorphs. For example, nearly typical specimens of ab. *colliurensis* Gelin (where the inner marginal black streak is absent), and of ab. *reducta* Fritsch (where the inner marginal black streak and also the lower black spot are absent) have been found. One specimen of each sex of ab. *chariclea* Steph. (where the black areas on the wing tips are dusted with white) and several examples of the diminutive form ab. *minor* Ksien. have also been noticed. An illustration of one of the gynandromorphs has already been published (Gardiner, 1957). The other example was mostly male but with a partial female forewing.

It may be concluded that in this culture, which has produced about 150,000 adults, the total number of aberrant forms is certainly very low, though inevitably some will have escaped notice.

Size and weight.

Without considering here the factors which influence the size and weight of the adult insects, a few figures may be given for insects taken from the general cultures. At various dates three batches of 25 insects have been selected at random. The average weights of the males were 135.5, 142.0 and 147.4 mg. with corresponding average wing spans of 58.4, 58.4 and 58.7 mm. (lowest 55.0 mm., highest 63.0 mm.). The average weights of the females were 137.6, 146.0 and 153.9 mg. with corresponding wing spans of 58.1, 56.3, 59.5 mm. (lowest 54.0 mm., highest 65.0 mm.). Frohawk (1934) does not give details of the weight of wild specimens but found that the wing span of males was 63.5 mm. and that of females 76.2 mm. The laboratory-bred insects were therefore smaller than the wild specimens measured by Frohawk.

Length of life.

It is only possible here to make a very general statement about the length of life of the adults in the culture. It is very much dependent on how well the insects feed (to be considered in a later paper), on temperature, and on atmospheric humidity. With these reservations it can be said that adults in the stock cage usually live for between 5 and 36 days, though not many survive to the end of this period.

Sex ratio.

At various times, over a period of three years, eight cages were selected from the culture and all the emerging insects, including crippled specimens, were sexed and counted. The sex ratio, males to females, varied from 0.84 to 1.35. The average ratio for the 2,687 insects counted was 1.05.

Mating.

To maintain a strong culture it is obviously necessary that mating should occur readily, and in fact caged insects usually copulate without difficulty except when daylight is excluded. It is nevertheless clear that many factors do influence the readiness with which mating occurs, and some of these have been investigated. They can be divided into two groups: those associated with the environment, and those associated with the insects themselves.

The influence of environmental factors on mating.

Mating at various temperatures.—A suitable temperature is a fundamental requirement for mating, but no attempt has been made to determine the exact maximum and minimum temperatures beyond which fully conditioned insects will not mate. It has, however, been observed that mating does not occur at 15°C. and that between 20 and 35°C. pairs form readily.

In order to compare mating at 20 and 30°C., insects, which had emerged between 5 p.m. and 10 a.m. the following morning in dim light in a constant-temperature room at 25°C., were sexed and separated into two cages of males and two of females. For the first experiment the insects were held at this intermediate temperature for two to three days, so that for the mating test some insects were warmed and others cooled. To avoid this difference in treatment the insects for the second experiment were first held for one to three days at 25°C. to mature and then for two days at 12.5°C., so that before mating both groups were warmed to the test temperature.

At the end of these preliminary treatments the insects were transferred in their cages to large-type mating cages (40 × 30 × 36 in. high), one at 20 and the other at 30°C. After being preconditioned for one hour to these temperatures, 20 of each sex were released into the main cage. As the pairs formed they were removed and replaced by insects from the small storage cages. The tests were continued until 20 pairs had formed or for 60 minutes, according to which condition was satisfied first. The results obtained are shown in Table I.

TABLE I.

Comparison of the rate of mating of 20 individuals of each sex of *P. brassicae* at 20 and 30°C. given various pre-test temperature treatments.

Period for which insects kept at 25°C. (days)	Subsequent temperature treatment (°C.) for 2 days	Age when tested (days)	Test temp. (°C.)	Number of pairs formed after minutes :							
				5	10	15	20	30	40	50	60
0-1	25	2-3	20	0	0	1	2	2	—	—	—
			30	4	7	10	15	20	—	—	—
1-3	12.5	3-5	20	1	1	1	1	1	2	2	2
			30	2	3	3	4	6	8	9	13

It can be seen that mating occurs more readily at 30 than at 20°C. and that this is true for insects brought to these temperatures from the intermediate temperature of 25°C. or from 12.5°C., which is colder than either.

In connection with the effect of temperature on mating, it seemed possible that within the range 20 to 30°C. a falling temperature might inhibit mating and a rising temperature stimulate mating. The procedure employed to test this idea was as follows: A batch of insects reared at 25°C. was divided into two small cages (12 in. cube) of males and two cages of females. One cage of each sex was conditioned for one hour to 20 and the other to 30°C. by standing it in one of two large mating cages (each 40 × 30 × 36 in. high) at the required temperature. After one hour, 20 of each sex were released into other small cages standing in the large cages and the temperatures were allowed to rise or fall by 10°C. over half an hour. As pairs formed they were replaced so that 20 potential pairs were always present in the mating cages.

TABLE II.

Two experiments on the mating of *P. brassicae* in rising and falling temperatures. Illumination >1,000 lumens/sq. ft.

Temperature change (°C.)	Number of pairs formed in 30 min.			
	First experiment		Second experiment	
	Below 25°C.	Above 25°C.	Below 25°C.	Above 25°C.
Rising from 20 to 30	5→11		7→8	
Falling from 30 to 20	2←12		2←8	

After 15 minutes, both cages were at 25°C. and the number of pairs formed above and below this temperature are shown in Table II.

It can be seen that pairing was not prevented by exposing the insects to a rapidly falling temperature though there is some evidence, especially from the second experiment, that it exerts a depressing effect. This question has not been more fully investigated as the chief concern was to show whether or not the adults were likely to be sensitive to the small, slow fluctuations of temperature which occurred during various mating tests.

In the course of these experiments it was observed that the length of time for which the pairs remained in copulation varied with the temperature. In some specially arranged tests, the insects were allowed to mate at 20, 25 and 30°C., and the pairs were kept at the same temperature or transferred to 15°C. after they had formed. The results obtained are shown in fig. 1. As would be expected, copulation lasted longer at lower temperatures. Occasionally pairs which formed in the late part of the afternoon did not separate until the following morning.

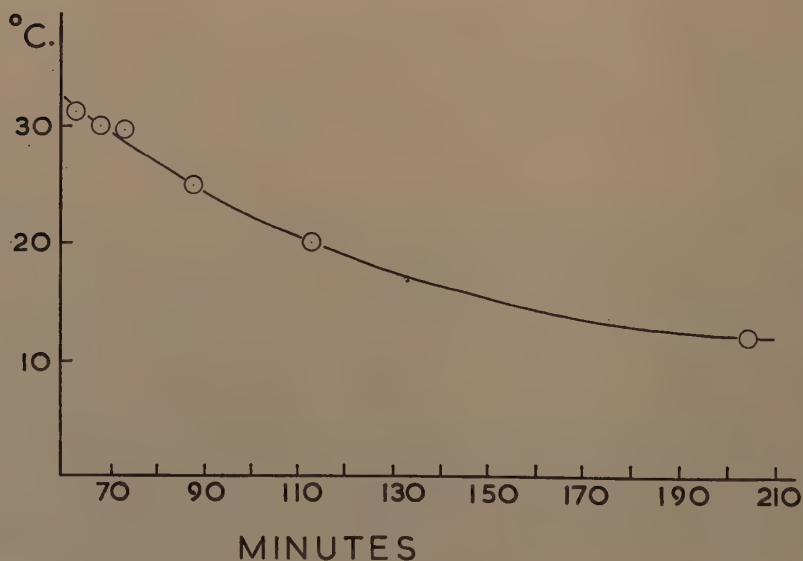


Fig. 1.—The average duration of copulation for *P. brassicae* at various temperatures between 15 and 30°C. (Insects at 15°C. were transferred to this temperature after the pairs had formed.)

It is evident from the foregoing results that *P. brassicae* will mate readily after exposure for short periods to 12.5°C. and the influence of longer periods at this temperature was investigated, as storing in the cold provides a convenient way of prolonging the life of unfed adults.

A batch of insects was kept at 12.5°C. and 60 per cent. relative humidity in a dim fluorescent light until they were 7 to 10 days old. They mated readily when transferred to 29°C., and all the eggs laid were fertile. In another batch, after a storage period of 19 to 20 days, most of the males had died and those which survived failed to mate. The females, however, mated with males 7 to 10 days old, but the eggs laid showed a marked drop in fertility and the hatch of various batches ranged from 10 to 80 per cent.

Although insects which have been stored in the cold in this way for 7–10 days are satisfactory for maintaining the culture, it would probably be unwise to use them for experimental purposes.

Mating in daylight of various intensities.—Adults of *P. brassicae* will not mate in the dark, but certain batches mate at low light intensities. Mating in relation to daylight intensity has been investigated independently and also in connection with the experiments in artificial light, which will be discussed later.

Unless stated otherwise, the adults for the experiments described in this section emerged in a constant-temperature room at 25°C. and were kept there, in fluorescent light, at 50–100 lumens/sq. ft., until used in the experiments. Under these conditions pairs never formed.

For mating tests at low daylight intensities, ten of each sex were transferred to a cubic-foot cage. This cage was placed within the large-type cage which was warmed to 28°C. and shaded with grey cloth so as to give the desired level of illumination. The following results were obtained:

At 150 lumens/sq. ft. daylight, six pairs formed in 24 min.

At 100 lumens/sq. ft. daylight, six pairs formed in 53 min.

While it is clear, therefore, that *P. brassicae* can mate at low daylight intensities, not all batches of insects will in fact do so, and, within the same batch, mating

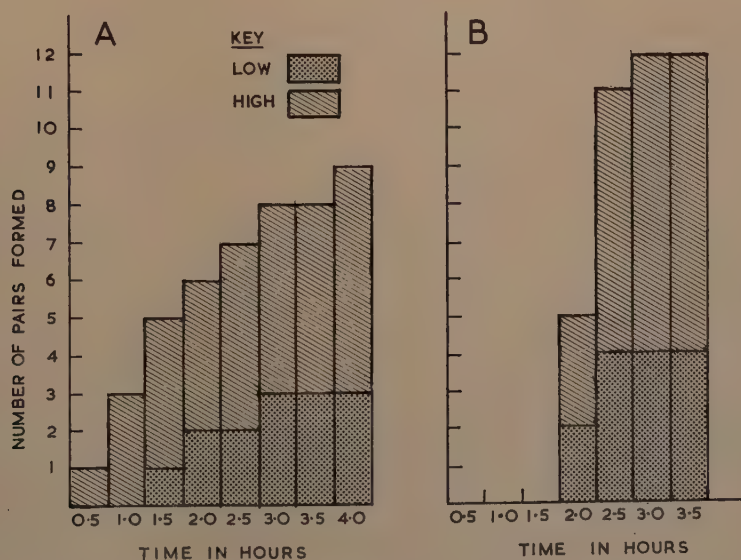


Fig. 2.—Comparative tests showing the mating of two batches of *P. brassicae* at two illumination levels in each case. Experiment A. Insects 2 to 26 hours old. Illumination levels: low (200 lumens/sq. ft.); high (1,600 lumens/sq. ft.); temperature, 30°C. Experiment B. Insects 2 to 50 hours old. Illumination levels: low (100 lumens/sq. ft.); high (1,600 lumens/sq. ft.); temperature, 30°C. In both experiments the stippled areas represent the results with the low level of illumination while the cross-hatched areas represent the results at the high level of illumination.

is always slower at low than at high light intensities. This is illustrated by the results of the two experiments shown in fig. 2. The air temperature in each of the mating cages was controlled so that it fluctuated less than $\pm 1^\circ\text{C}$. and on the average the cage with dim light was a little warmer than the cage with bright light.

The influence of daylight quality on mating.—The spectral energy distribution of daylight varies at different times of the day, but at adequate illumination levels mating occurred quite readily at any time from early morning to late afternoon.

When it was found that insects did not mate satisfactorily in artificial light, tests were carried out in an effort to determine whether, in daylight tests, they were responding to polarised light from a particular region of the sky. No evidence was obtained to support this suggestion, but it proved to be difficult to arrange satisfactory test conditions and the results should not be regarded as conclusive.

Mating in various artificial lights.—The intensity of daylight and the temperature varied in the glasshouse where the stock culture was maintained, and in order to rear *P. brassicae* under standard conditions attempts have been made to establish a culture in a constant-temperature room with controlled humidity and artificial lighting. The results were unsatisfactory since the insects were reluctant to mate in the artificial lighting provided. The reason for this is not understood and presents an interesting problem.

There are three obvious ways in which light from artificial sources may differ from daylight. It will tend to be lower in intensity, of different spectral energy distribution and much more directional, with a strong gradient in intensity. Attempts have been made to investigate these factors using light from the following types of lamps:

1. 500-watt incandescent tungsten filament gas-filled in clear glass;
2. 80-watt 'daylight' fluorescent, MCF/U;
3. 400-watt mercury vapour, MA/V;
4. 140-watt sodium vapour, SO/H.

Some information about the distribution of energy in the various spectral regions emitted by these lamps is presented in Table III (B. S. Cooper, personal communication, 1960) and in the following paragraphs.

The energy radiated by the tungsten lamp is emitted in a continuous spectrum over the visible range and also extends far into the infrared. Most of the energy is concentrated in the red and infrared regions. The light is deficient in energy in the blue region and contains practically no ultraviolet.

The spectral energy distribution of the energy from a daylight fluorescent lamp also differs significantly from sunlight, as may be seen from Table III. It is notably deficient in the near infrared region. The energy emission in the visible range forms a continuous spectrum with peaks of high emission corresponding with the main lines of the mercury discharge. In general, the energy distribution of the light from this lamp resembles that of daylight much more closely than that of the light from a tungsten lamp and it also contains a small proportion of near ultraviolet radiation in the wavelength range 3100 Å to 4000 Å. This amounts to between 5 and 10 per cent. of the visible radiant energy. In direct sunlight, the corresponding proportion of near ultraviolet energy is about 12 per cent. of the visible radiant energy.

The high-pressure mercury-vapour lamp emits its energy in a series of monochromatic radiations without appreciable continuum. The strongest spectral lines lie at 3650 Å (long-wave ultraviolet), 4046 Å and 4358 Å (blue), 5461 Å (green) and 5790 Å (yellow).

The sodium-vapour lamp produces monochromatic yellow light, almost all its energy in the visible range being concentrated in a doublet at 5890 and 5896 Å.

In the case of all lamps, the hot envelopes also emit infrared radiations.

Tungsten light.—From the experiments with daylight of different intensities it was clear that a high illumination level favoured mating. High-wattage tungsten lamps are an obvious source for achieving high intensity lighting and these were tested first.

Apart from the results in one early test, the mating response to tungsten light was unsatisfactory.

In the successful test, 15 individuals of each sex, which had been bred throughout in the constant-temperature room, were put together, when 2-3 days old, in a cage measuring 13 × 13 × 18 in. high. Two 500-watt tungsten lamps above the

TABLE III.

Total radiant power falling on unit area, and proportion in various spectral regions, for different light sources at equal illumination level of 500 lumens/sq. ft.

Source	Radiant power falling on unit area when illumination = 500 lumens/sq. ft.	Percentage in various spectral regions			
		approximately 3000Å-4500Å	approximately 4500Å-7500Å	approximately 7500Å-14000Å	> 14000Å
		near ultra-violet and violet	remainder of visible spectrum	near infra-red	longer infra-red (absorbed strongly by water)
	milliwatts/cm. ²				
Direct sunlight ..	3.7	12	41	31	16
Tungsten filament ..	23.3	2	11	26	61
Fluorescent (daylight)	4.4	8	34.5	1.5	56
High-pressure mercury (MA)	8.0	6	11	3	80
Sodium	3.9	1	29	4	66

cage gave a maximum illumination in the cage of 280 lumens/sq. ft. and the maximum temperature was 29°C. Within two hours, three pairs formed and in the following three days five further pairs were observed. In fact it is likely that other females mated, since, from the fifth to the eleventh day, over 850 fertile eggs were laid daily.

Three attempts to repeat this test under apparently identical conditions were unsuccessful. At the most, only one pair formed in up to three hours, yet when the same insects were transferred to daylight, at the same intensity, 5 to 11 pairs formed within ten minutes.

Although these repeat tests were unsuccessful, the fact that mating was satisfactory in one test and that occasional pairs formed in other tests suggested that some modification of the conditions might lead to consistently high levels of mating. The following changes were tried without, however, producing any significant improvement:

1. The illumination was increased to 480 lumens/sq. ft. by using four 500-watt lamps.
2. The light was diffused through glass and paper screens.
3. The radiant heat from the lamps was reduced by a water screen, two in. deep.

4. The light was made less directional by increasing the proportion of reflected light by surrounding the cage with white screens.
5. Insects of various age ranges from 1-3 days to 4-5 days old were tested.

In the case of these tests also, the capacity of the insects to mate when transferred to daylight was checked. The procedure was as follows: The mating cage was carried to the glasshouse in a black box and placed in a white enclosure open at the top and on one side. This was screened so as to give the same light intensity in the cage as in the test. The glasshouse was always warmed and sometimes humidified to the test conditions. In all cases, in a short time the insects mated in satisfactory numbers. The results of some of the experiments carried out in this way are shown in Table IV.

TABLE IV.

Mating of *P. brassicae* in overhead tungsten lighting.

Age of insects (days)	Tungsten light				Daylight			
	Intensity (lumens/sq. ft.)	Temp. (°C.)	Time (hr., min.)	No. of pairs formed	Intensity (lumens/sq. ft.)	Temp. (°C.)	Time (min.)	No. of additional pairs formed
1-3	200 ¹	30	0.45	1	200	30	14	5
1-3	200	32	0.45	0	150	30	18	10
2-3	200	30	2.15	0	200	27	9	8
3-4	200	30	2.45	1	200	27	11	11
4-5	200	31	1.30	1	200	30	44	5
2-3	400 ²	30	5.15	1	400	29	10	7
2-3	400	28	2.0	1	400	26	60	11

The table gives details of the number of pairs formed in tungsten light out of a possible 15 and of the additional pairs formed on transferring the cages of test insects to daylight.

¹ In all tests at 200 lumens/sq. ft. the unscreened light from two 500-watt lamps was used.

² In the tests at 400 lumens/sq. ft. the light from four or six 500-watt lamps screened through greaseproof paper and a water layer, two in. deep, was used.

As the tungsten lamps were usually arranged in a reflector with the filaments between one and two ft. from the top of small mating cages (13 × 13 × 19 in. high) the light was very directional and the intensity gradient steep. It seemed possible that this kind of lighting was unfavourable for mating, and an arrangement giving bilateral illumination was tested. A mating cage (1 cu. ft.) covered with mosquito netting was placed in an air-conditioned cabinet with glass side-walls. Two tungsten lamps were placed outside the cabinet on each side so that they illuminated the test cage through the cooled glass walls. The illumination in the mating cage was 340 lumens/sq. ft. (with the photocell facing laterally) and the temperature between 31 and 33°C. Two tests gave a reasonably satisfactory level of mating but all the others gave a very low response although the insects mated when transferred to daylight of the same intensity.

It may be concluded from these tests that certain insects will mate in tungsten light but none of the variations tested produced a consistently high level of response. The reason for the better response of certain batches is not understood.

Fluorescent light.—The spectral energy distribution of light from tungsten lamps differs from daylight, being relatively deficient in energy at the blue end of the spectrum, and it was thought that this factor might have been responsible for the low level of mating in tungsten light. 'Daylight' fluorescent tubes were tested instead, since the light from these lamps matches the light reflected from

the sky more closely. It could therefore reasonably be expected to be more favourable for stimulating mating.

Several batches of insects cultured between July 1955 and June 1956 were tested with bilateral illumination in the air-conditioned cabinet previously used in the tests with tungsten illumination. The cabinet was fitted with five fluorescent tubes on each side and the light level in a mosquito-netting mating cage was about 500 lumens/sq. ft. with the photocell facing laterally. Fifteen adults of each sex were used in each test.

The results obtained were again erratic and some batches of insects entirely failed to mate. On the whole, however, much more consistent results were obtained than with tungsten lamps. In a total of 12 mating tests, eight gave four or more pairs, and these results are given in detail in Table V. In the remaining four tests, where the conditions were apparently the same, no pairs formed.

It may be concluded that most batches of *P. brassicae* will mate in bilateral fluorescent light but that it is a considerably less satisfactory stimulant for mating than daylight of the same intensity.

TABLE V.

Mating of *P. brassicae* in bilateral fluorescent light.

Age of insects (days)	Fluorescent light				Daylight			
	Intensity (lumens/sq. ft.)	Temp. (°C.)	Time (hr., min.)	No. of pairs formed	Intensity (lumens/sq. ft.)	Temp. (°C.)	Time (hr., min.)	No. of additional pairs formed
1-2	450	30	3.55	6	—	—	—	—
1-2	450	30	6.20	5	—	—	—	—
1-2	450	29	4.45	9	150	30	4.45	9 ¹
2-3	500	29	5.15	4	1000	29	0.15	10
2-3	480	30	4.15	6	200	30	0.6	5
2-3	450	30	4.15	5	250	29	1.0	1
2-3	450	29	2.0	5	—	—	—	—
2-3	450	30	6.45	5	200	28	0.20	7

The table gives details of the tests in which four or more pairs formed out of a possible 15 and, unless indicated otherwise, the number of additional pairs formed on transferring the cages of test insects to daylight.

¹ These insects were a separate batch taken from the same stock cage as those used in the fluorescent light.

Mixed light sources.—It is obvious from the foregoing tests that neither tungsten light nor fluorescent light is as satisfactory as daylight for stimulating mating. The spectral energy distribution of both sources differs from daylight, as has been pointed out, and attempts were made to correct these deficiencies by using combined light sources. For example, a tungsten lamp will supply the near infrared radiation in which the light of a fluorescent lamp is deficient, and the mercury-vapour lamp the ultraviolet radiation lacking in the light of a tungsten lamp.

Experiments were set up with overhead and bilateral arrangements of the lamps. In the latter case the constant-temperature cabinet mentioned previously was employed. With the overhead arrangement there was no screen between the lights and the mating cage but with the bilateral arrangement there were three glass plates between the insects and the lamps.

Representative results of some of the many experiments with lights from mixed sources are given in Table VI. It can be seen that none of these light combinations is as satisfactory for mating as daylight in the glasshouse to which the insects were subsequently exposed.

TABLE VI.

Mating of *P. brassicae* in light from mixed sources arranged overhead or bilaterally.

Source ¹	Direction	Artificial Light				Daylight			
		Intensity ² (lumens/ sq. ft.)	Temp. (°C.)	Time (hr., min.)	No. of pairs	Intensity (lumens/ sq. ft.)	Temp. (°C.)	Time (hr., min.)	No. of addi- tional pairs
3T, 1Hg	Overhead	600	32	4.30	0	300	28	1.45	4
4T, 2Hg	"	500	29	5.0	0	400	27	2.7	10
4T, 2Hg	"	500	29	5.0	0	500	29	1.30	9
1T, 2Hg, 1 Na	"	500	28	5.0	0	250	28	1.0	5
4T, 2Hg	"	300	28	2.30	0	400	29	1.15	6
4T, 4F	Bilateral	600	29	1.15	2	400	28	1.15	6
4T, 4F	"	600	29	3.15	0	300	30	0.55	4

The table shows the number of pairs out of a possible 15 formed in artificial light and the additional number formed on transferring the cage to daylight either on the following day or immediately, as in the last three tests. The insects were 2-3 days old.

¹ The total number of lamps in use is given. F='daylight' type fluorescent, Hg=mercury vapour, Na=sodium vapour, T=tungsten.

² The illumination level was measured facing the light source in all cases from the centre of the mating cage.

Mating and atmospheric contamination.—The mating tests in artificial light just described were carried out in a constant-temperature room in the main laboratory building whereas those in daylight were carried out in a glasshouse.

The air in a laboratory tends to be contaminated with variable amounts of fumes from chemicals and burners and also with traces of coal gas. The latter is often present in sufficient concentration to cause epinasty in sensitive plants. It therefore seemed important to demonstrate at this point that mating in the tests in artificial light was not being inhibited by substances contaminating the air of the constant-temperature room.

In the first tests, daylight was admitted to the constant-temperature room. Under these conditions, adults in a cage standing near the window mated readily. For other tests in the glasshouse the insects were released into mating cages in which the air was much more heavily contaminated with coal gas or the fumes of a burning oil lamp than ever occurred in the laboratory. In these tests also the insects mated readily.

It may be concluded that the insects in the tests which were conducted in the constant-temperature room were not inhibited from mating by substances contaminating the air.

Mating at low and high relative humidities.—At the conclusion of the tests on mating in artificial light, the insects were transferred to a glasshouse to establish that they would mate in daylight of the same intensity. For these tests it seemed necessary that the temperature and humidity in the glasshouse should be the same as in the constant-temperature room or to show that the changes involved were not significant. It has already been shown that the mating response was affected by temperature, and this was controlled. The humidity in the

glasshouse was much less readily adjusted, and, instead of attempting to do this regularly, an experiment to determine the influence of humidity on mating was set up.

The male and female insects for these tests were kept in separate cages in the constant-temperature room at 25°C. and 55 to 60 per cent. relative humidity until they were 2-3 days old. On the morning of the test, the stock cages were sprayed lightly with water so that the insects could drink all they required. This was a necessary preliminary as otherwise the insects in the high-humidity test cage remained motionless drinking the water droplets from the spray used to humidify the cage. One hour later, twenty of each sex were released into each of two large mating cages. The humidity in the drier cage was at the same level as in the glasshouse, while the other cage was brought to a higher level by screening it with sheets of glass, spraying with water, and injecting steam. During the actual mating period the humidity was maintained with steam. As the pairs formed they were replaced by other insects.

The results set out in Table VII show that the insects mated about equally readily over the range of relative humidity 30-50 per cent. but that, in comparison, mating was much less frequent above 70 per cent.

As the relative humidity in the glasshouse in day-time was usually in the range 30-60 per cent., it was concluded that no effort need be made to adjust it for the purpose of the mating tests.

TABLE VII.

The influence of atmospheric relative humidity on the mating of *P. brassicae*.

Daylight intensity during test period (lumens/sq. ft.)	Duration of test (min.)	Low humidity			High humidity		
		R.H. (%)	Temp. (°C.)	Pairs formed	R.H. (%)	Temp. (°C.)	Pairs formed
>1000	14	30	30-31	34	70-80	30-32	6
400-900	27	32	30-33	36	70	30-32	6
600-1000	13	32 ¹	30	26	70	30-31	2
900	6	32	29-30	9	50	28-29	11
>1000	18	29	28-31	10	54	29-31	16

The tests were done in April 1958 with insects 2-3 days old.

¹ The relative humidity in this cage was increased to 70-80 per cent. after the 13-minute observation and the test was continued at this level for a further 27 minutes. Only one pair formed at the raised humidity.

Mating in cages of various sizes.—When the culture of *P. brassicae* was established it was assumed, without evidence, that a large cage (40 × 30 × 36 in. high) would be more favourable for mating than a smaller cage. One reason for this supposition was that the males frequently begin to pursue the females in flight and in larger cages flights tend to be of longer duration giving more opportunity for the pursuit to begin. But males also approach resting females, and in practice it has been found that pairs form in small cages, 12 in. cube or 13 × 13 × 15 in., as readily as in the larger cage.

In a comparative experiment, 12 individually marked males and females, 2-4 days old, were introduced into a large and a small cage. The times at which the pairs formed and the periods in copulation were noted.

There is a disadvantage in carrying out the test in this way since the population density (and so presumably the number of encounters between males and females) is greater in the small than in the large cage. This objection has been ignored in

the present test since it was intended merely to show that a small cage was satisfactory for mating, and the influence of population density will be considered in the next section.

The results of a typical experiment carried out in this way are shown in fig. 3. It can be seen that pairs formed just as readily in the small cage as in the large cage. Such small differences as exist between the behaviour of the adults in the two cages are probably due to the higher temperatures which prevailed in the small cage during the early part of the test.

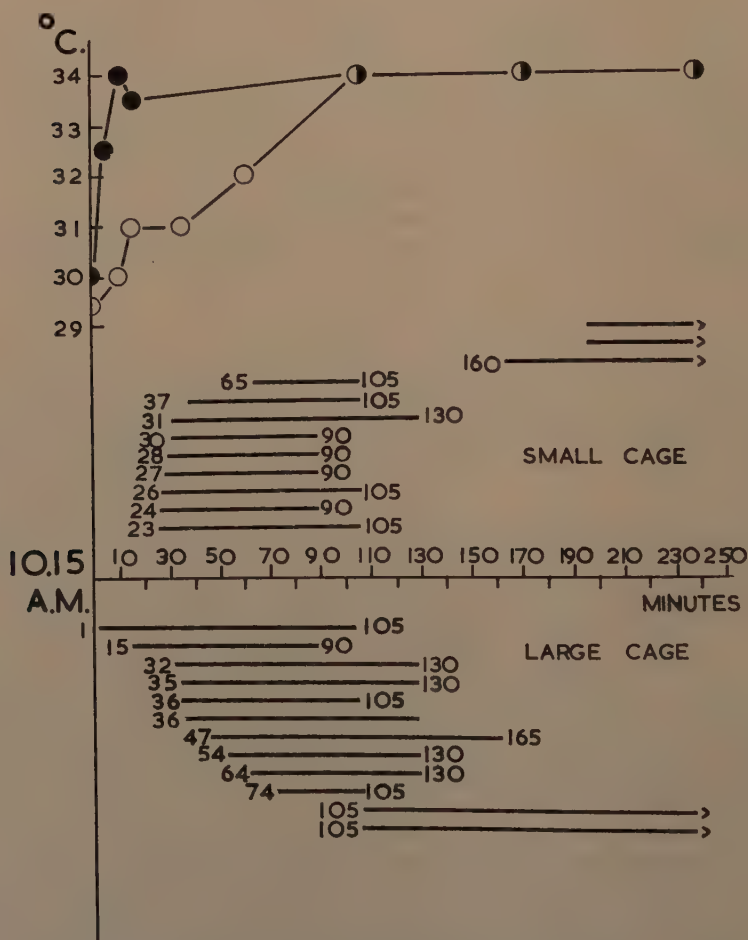


Fig. 3.—Mating of *P. brassicae* in large cages (40×30×36 in. high) and small (12 in. cube). Twelve individually marked insects of each sex, 2–4 days old, were used. The times at which the first nine pairs formed and the duration of the copulations are shown in each case. The fluctuations in temperature in the two cages over the same period are shown by the graphs at the top of the figure.

Mating at high and low population densities.—The influence of population density in a mating cage on the number of pairs formed in a given time has been investigated using the large (40×30×36 in. high) and the small (12 in. cube) cages.

Comparisons were made between the numbers of pairs formed when five or 20 adults of each sex were released simultaneously into cages of the same size. The two cages stood side-by-side and they were so orientated that the light and temperature were the same in both. As the pairs formed they were replaced by fresh males and females and the test was continued until the stock of adults was exhausted.

TABLE VIII.

The effect of the population density in mating cages of two sizes on the formation of pairs.

Age (days)	Temp. (°C.)	Light intensity (lumens/sq. ft.)	Duration of test (min.)	Percentage of pairs formed in cages maintained at :	
				5 of each sex	20 of each sex
1-2	31	>1000	14	Small cage	
1-2	30	>1000	29	58	43
				44	43
2-4	28	800	12	Large cage	
2-3	29	400	18	17	50
				37	56

The results of experiments with four different batches are given in Table VIII. In the small cages, where the population density was either 5 or 20 of each sex per cubic ft., the insects mated about equally readily at both densities. In the large cage, populations of 0.2 adults of each sex per cubic ft. (5 of each sex in the cage) resulted in a definitely lower percentage of matings, in a given time, than a population of 0.8 of each sex per cubic ft. (20 of each sex in the cage). It may be concluded that the rate at which pairs formed was fairly constant over the population density range 1 to 20 adults of each sex per cubic ft.

The influence of biological factors on mating.

Mating behaviour.—When virgin insects of suitable age are brought together for the first time in daylight at normal temperature, mating begins almost immediately. The male may pursue the female in flight and the two land together and pair so quickly that the exact sequence of events is difficult to follow. But rather more frequently the males land on, or beside, the resting females. In the former case the male twists his abdomen laterally towards the female and inserts the tip between her folded wings from below, to make contact with the genital opening. When the union is established the male swings round behind the female so that the pair are in line, with the folded wings of the male outside those of the female and partly overlapping them on both sides. If disturbed the pair may fly to another resting place without separating. In such a flight only the male uses his wings.

If the female is not prepared to mate she avoids the male in flight and if he approaches her at rest she spreads out her wings and curves her abdomen dorsally. At first this movement was interpreted as an invitation to the male, but in fact it is a rejection posture and effectively prevents the male from making the normal approach to the female which has just been described. It may also have some warning significance since it is quite frequently adopted by a resting male when disturbed.

Under caged conditions the sexually active male does not always immediately distinguish a female from a male and he may pursue a male until the latter lands and adopts the rejection posture just described.

Mating in relation to the age of the adults.—A few preliminary observations had shown that the adults did not mate readily until a day or more after emergence and, as it was important to know the optimum age for mating in connection with the other tests, experiments were designed to investigate this point.

In preparation for these tests, large batches of larvae, with more than the usual scatter in age range, were bred together so as to give pupae from which adults would emerge over several days.

Two experimental procedures have been followed. In the first, two groups of adults, aged 1 to 18 hours and 49 to 66 hours, respectively, were compared in two of the large-type mating cages standing side-by-side under apparently identical conditions of temperature (30 to 32°C.) and illumination (400 to more than 1,000 lumens/sq. ft.). Twenty insects of each sex were put into the cages initially and these were replaced when they mated. After 90 minutes, seven pairs of the younger and 57 pairs of the older insects had formed. The experiment was repeated at 25 to 27°C. and 100 to 300 lumens/sq. ft. (the sky was overcast). After four hours, one pair of the younger and 18 pairs of the older insects had formed.

In the second method, insects of three or four age-groups were put together in the same large cage so that competition between the groups could occur. In this way it seemed that it would be possible to show whether the same age was optimum for males and females.

The adults emerging between 5 p.m. on one day and 10 a.m. on the following day were collected. The sexes were separated, and the insects of different ages and sexes were all marked with distinctive coloured spots, on the bases of the under surfaces of the hind wings, so that the age and sex of the insects which had paired could be recognised immediately. These insects were then stored, unfed, until required. The test was set up at 11 a.m. on the day on which the last group of insects was collected so that the test batches were 1 to 18, 25 to 42, 49 to 66 and 73 to 90 hours old.

Ten males of each age-group were first released into the cage and then, as rapidly as possible, ten females from each group. As the pairs formed they were replaced by insects of the same age.

Three tests were carried out by this method. Relatively few pairs formed between adults of certain age-groups while others mated rapidly, so that the stocks of these became exhausted and the tests then had to be discontinued. In order to obtain more reliable figures for the age-groups which mated reluctantly the results of the three tests have been combined in Table IX. This table therefore

TABLE IX.

Competitive mating test between males and females of *P. brassicae* of four different age ranges.

Age (hours)	Females			
	1-18	25-42	49-66	73-90
Males				
1-18	0	0	2	2
25-42	1	17	45	28
49-66	6	25	50	31
73-90	0	4	21	12

The figures in the body of the table show the numbers of pairs formed and the ages of the partners. Temperature range in tests 27 to 30°C. Illumination range 160 to 600 lumens/sq. ft.

represents the mating response of 568 insects including the 40 of each sex (10 of each age) remaining in the cage at the end of the test. Only apparently normal, active, insects were selected for the test but it was noticed that some of the insects in the stock of the oldest group, especially the males, were dying of starvation or desiccation. It is doubtless for this reason that comparatively few of these insects mated even when they appeared to be healthy.

The results show that it is unusual for males or females 1 to 18 hours old to mate. By the time the males are 25 to 42 hours old they mate almost as readily as when 49 to 66 hours old but the females become more ready to mate during this period. In making this comparison, however, it should be borne in mind that there is a tendency for the males in a culture to emerge, on an average, slightly before the females, so that in the test groups the true average age of the males may be slightly greater than that of the females. This, rather than earlier physiological maturity, might explain why the males appeared to be ready to mate before the females. To test this point, very large batches of insects would have to be reared so as to obtain the necessary numbers of males and females emerging over a much narrower time range than used in these experiments.

Frequency of mating.—Observations have been made on batches of marked insects to determine how frequently mating occurred. Two- to three-day-old insects which had been stored as separate sexes at 25°C. were allowed to mate in daylight. As soon as the pairs formed, ten were transferred to a large-type mating cage. For the rest of the day (from 12 noon to 5 p.m.) they were observed at half-hour intervals so that any further pairing which occurred would be detected. At 5 p.m. each day, the cage was covered with a black cloth to prevent further mating. This was kept on until 10 a.m. the following morning when observations were resumed.

Out of 20 females in two tests, only two mated a second time on the first day and one of these mated again on the second day—the only one to do so. No further pairs formed until the fifth day when two females mated. By the tenth

TABLE X.

Mating behaviour of five males with 15 females. The insects were 2-3 days old.

Male number	Times at which matings occurred							
	First hr., min.		Second hr., min.		Third hr., min.		Fourth hr., min.	
Exp. 1 ¹								
1	0	1	1	18	1	42	4	30
2	0	1	2	30	—	—	—	—
3	0	2	1	24	—	—	—	—
4	0	5	1	0	1	18	5	0
5	0	5	2	30	—	—	—	—
Exp. 2 ²								
1	0	7	5	0	—	—	—	—
2	0	11	1	1	3	30	4	0
3	0	13	1	5	5	0	—	—
4	0	25	—	—	—	—	—	—
5	1	1	—	—	—	—	—	—

¹ The temperature varied between 27 and 30°C. and the illumination level between 400 and 1,000 lumens/sq. ft.

² The temperature varied between 28 and 29°C. and the illumination level between 400 and 500 lumens/sq. ft.

day, 18 out of the 20 females had mated twice and, of these, four had mated three times and two four times.

The normal period between effective matings for females is therefore between six and nine days under these conditions. It seems possible that when mating was repeated on consecutive days it was because the first union was not fully effective.

Out of 20 males in the two tests, 14 mated either twice or three times in ten days. One mated four times, and two other males mated twice on the same day. From these results it was thought probable that the males would have mated more frequently had an excess of unfertilised females been present and, in further experiments, this was shown to be true.

Virgin insects, 2-3 days old, were put together in the large-type mating cages. Five individually marked males were used and 15 females, which were marked as the pairs formed. The results obtained in two experiments are shown in Table X. The males mated between one and four times during experiments which lasted five hours. In the first of these, 12 out of the 15 females mated, and in the second experiment, 9 out of 15 mated, some mating twice in each experiment. Possibly, had a greater excess of females been present, the males would have mated even more times. It would have been interesting to have tested this and also to have determined whether as many fertilised eggs were laid by females which were partners in the last copulations as by those fertilised first, but this has not been done. A preliminary experiment showed that the males were ready to mate again on the following day.

Diurnal rhythm and mating.—Mating commences in a stock cage of adults soon after the temperature and the light intensity reach satisfactory levels in the morning and new pairs continue to form all day. Apart from the fact that pairs tend to form as soon as satisfactory conditions are restored in the morning, there is no evidence that any particular time of the day is favoured for mating. In the case of insects which are brought out into daylight from the dim artificial light of the constant-temperature room, it has been found that they will mate readily at any time of the day provided the temperature and light intensity are satisfactory.

Discussion.

The experiments on factors influencing mating described in this paper form part of an investigation into the conditions which are important in maintaining a culture of *P. brassicae* in captivity. The results show that it is unlikely that difficulty will be experienced in getting the insects to mate provided they are kept in daylight at a suitable temperature and that they have reached the correct age.

In artificial lighting, mating occurred much less readily than in daylight. Fluorescent, 'daylight'-type lamps gave the best results, and it seems probable that the level of mating in this light would have been sufficient to maintain the culture. At the time that this paper was being written, it was learned that a culture of *P. brassicae* had been maintained throughout the winter in a windowless room. The cage was illuminated by two 40-watt fluorescent tubes. Mating was seldom seen among the hundreds of adults, but there were always plenty of eggs (P. T. Walker, personal communication, 1960). From observations made on various stocks, it has been concluded that strains adapted to feeding on artificial flowers are selected under the conditions of breeding in the adult cages. This subject will be considered in a later paper, but the observation suggests that strains which mate readily in artificial light could also be selected. With daylight-adapted insect stocks, however, the more certain way of maintaining a culture in a conditioned room would be to admit controlled amounts of daylight—sufficient to stimulate mating but not enough to upset the other conditions.

The reason why the artificial illuminants were less satisfactory than daylight

for stimulating mating has not been definitely established. The deficiency was not in intensity, but it seems probable that it was connected with the spectral energy distribution of the light sources which in all cases differed significantly from daylight. This suggestion is supported by the observation that the fluorescent light, which most closely resembled daylight in spectral energy distribution, was the most successful in inducing mating. It is also important that the light should not be too directional.

Consideration has been given to the suggestion that the artificial illuminants were unfavourable for mating because the high proportion of infrared which they radiated led to the insects being overheated (Table III). For example, sunlight has about 16 per cent. of its radiant power at wavelengths above 14000 Å, whereas, with all other sources, the corresponding values are upwards of 50 per cent. and may be as high as 80 per cent. Again, sunlight has about 31 per cent. of its radiant power in the near infrared region whereas all the lamps tested except the tungsten emit very little energy in this range.

When tungsten light was screened with a layer of water, two in. deep, to cut down the proportion of infrared radiation of wavelength above 14000 Å, the light was still unsatisfactory for mating. Furthermore, a low intensity of tungsten light, to supply near infrared radiation, combined with fluorescent light did not improve the results obtained in comparison with fluorescent light alone. Finally, the 500-watt tungsten lamp used over the adult stock cage enhanced rather than depressed mating activity on overcast days (David & Gardiner, 1952). The conclusion of Digby (1955) who found that "for insects of breadth greater than 0.3 cm. spectral composition of radiation over the normal sunshine range was of negligible importance to the temperature excess" of the insect lends further support to the belief that it is not the relatively high percentage of infrared radiation from the artificial light sources which renders them unfavourable for stimulating the mating of daylight-adapted insects.

As an explanation of the inferiority of the fluorescent lamps compared with daylight in stimulating mating, the possibility that the 50-cycle flicker of the light was having a depressant effect was considered. No difference could be found, however, in the mating response of batches of insects exposed to the light of three double-tube units connected to a single phase as compared with three double-tube units each connected to a separate phase. Neither arrangement was as effective as daylight of similar intensity.

Taken together, these results suggest the conclusion that daylight is the most satisfactory form of illumination for maintaining a culture, but it seems certain that stocks adapted to mating in fluorescent light could easily be selected. It would be interesting to see whether, by adding light in certain wavelengths, the light from fluorescent tubes could be made as satisfactory as daylight for promoting the mating of adults of *P. brassicae* accustomed to mate in daylight.

Summary.

As part of a general study of the conditions required for satisfactory maintenance of *Pieris brassicae* (L.) in laboratory culture, an investigation was made of the factors affecting mating behaviour. Notes are given on certain characteristics of the culture from which the experimental material was drawn.

It was established that *P. brassicae*, over one day old, mated readily at temperatures between 20 and 32°C. provided the daylight illumination was above about 200 lumens/sq. ft. Mating occurred more readily at the higher temperatures and illumination levels than at the lower. It was depressed at very high atmospheric humidities. The size of the cage in which the insects were held was not critical, and pairs formed readily in cages as small as one cubic foot. The population density in the cage was also varied over wide limits without significantly influencing the rate at which pairs formed.

Mating took place much less readily in the artificial lights which were tested than in daylight. The most satisfactory light was found to be bilaterally arranged 'daylight'-type fluorescent lamps. The level of mating in this light would probably have been sufficient to maintain a culture but it was far lower than in daylight of the same intensity.

After mating, the females do not usually pair again for five or more days. The males, however, mate more frequently and will often pair several times in the same day.

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OBSERVATIONS ON NATURAL MORTALITY, PARASITES AND
PREDATORS OF WHEAT BULB FLY, *LEPTOHYLEMYIA*
COARCTATA (FALL.).

By RONALD M. DOBSON *

E.M.A.

Rothamsted Experimental Station, Harpenden, Herts.

Knowledge of natural mortality of wheat bulb fly, *Leptohylemyia coarctata* (Fall.), is fragmentary, and losses during the complete life-cycle have not been studied. Dobson, Stephenson & Lofty (1958) found from cage experiment that at least 4.7 per cent. of the eggs present in mid-March survived to produce flies, and in field experiments Raw (1960) observed survival varying from 2.7 to 17 per cent. between the end of oviposition and pupation.

Losses to eggs varying from 90 per cent. (Bremer, 1929; Gough, 1947) to 18-27 per cent. (Raw, 1960) have been recorded and attributed partly to "non-viability" and partly to predation. Many larvae perish because they fail to find food-plants, either when they are newly hatched or when they are moving to fresh shoots (Gough, 1947), and both F. Raw & J. R. Lofty and D. B. Long (*in* Mellanby, 1958) and Raw (1960) showed that the proportion of survivors is affected by plant density.

Losses of pupae were estimated as 78 per cent. by Dobson, Stephenson & Lofty (1958) and, although this figure was thought to be an overestimate, a loss of at least 66 per cent. was apparent. Parasites kill some pupae (Van Miegroet, 1950; Bardner & Kenten, 1957) and Van Miegroet recorded between 18 and 27.35 per cent. parasitisation of populations in Belgium.

Life-span of the adult fly has been studied (Dobson & Morris, 1961) but little is known of causes of mortality. Predators (Bardner & Kenten, 1957) and entomophagous fungi (Gough, 1947; Bardner & Kenten, 1957; Long, *in* Mellanby, 1958) cause some deaths.

The present paper discusses mortality during the pupal stage with special reference to parasitism and describes simple experiments with predators. In 1957 and 1958, the observations were incidental to the main line of work, but from the summer of 1959 were its main purpose. [This, however, was curtailed after a few months because the writer left Rothamsted in November 1959.]

TABLE I.

Losses amongst pupae kept in pots of soil at Rothamsted in 1957 and 1958.

Year	Total no. of pupae collected	No. of flies emerging	No. of pupae parasitised	No. of pupae found dead	No. seen to be removed by ants	No. of pupae missing
1957	900	296	90	46	3	465
1958	4,010	2,240	206	53	0	1,511

* Work completed at present address: Zoology Department, University, Glasgow W.2.

TABLE II.
Losses amongst pupae obtained from Whittlesey and Peterborough during 1959.

Site	Date of collecting	No. of pupae collected	No. of flies emerging	Parasites				Died due to unknown causes
				<i>Aleochara inconspicua</i>	<i>Aleochara bipustulata</i>	<i>Aleochara?</i>	<i>Phygadeuon trichops</i>	
Whittlesey	May 5	461	357	3*	0	10	0	91
	12	467	374	11	7	3	0	72
	19	501	361	10	15	20	0	94
	26	468	347	5	17	7	1	91
	June 2	463	310	0	16	3	0	134
	Total	2,360	1,749	29	55	43	2	482
Peterborough	May 8	479	331	49*	0	20	0	78
	15	476	330	11	39	20	0	76
	22	464	302	11	59	9	0	83
	29	513	387	4	34	3	0	85
	June 4	509	332	1	69	3	0	104
	Total	2,442	1,682	76	202	55	0	426

* Underestimate

Mortality amongst pupae.

Observations during 1957 and 1958.

In 1957 and 1958, in the course of a study on the life-span and behaviour of adult flies in eastern England, many pupae from a wheat crop at Rothamsted were placed in soil in gauze-covered plant pots and kept in the field until flies ceased to emerge. The pots were then searched and any pupae remaining were examined directly for parasites (1957) or were counted and replaced so that parasites could emerge naturally (1958).

The results (Table I) show that, as in the field-cage observations reported earlier (Dobson, Stephenson & Lofty, 1958), losses were high, and that although parasites and predators accounted for some, most were unexplained. Clearly, more detailed observations were needed.

Two species of parasitic Hymenoptera were obtained during these observations. One, a Braconid, occurred as a single imperfect specimen in 1957 and could not be identified, the other, a Cynipid, affected about 10 and 5 per cent. of the populations in 1957 and 1958, respectively, and has been named *Trybliographa spaniandra* by Kerrich & Quinlan (1960).

Observations in 1959.

In 1959, a full-time study of mortality was started. As the Rothamsted population was sparse, pupae were obtained at weekly intervals for five weeks from two fenland sites, Whittlesey and Peterborough. Most of the pupae were placed singly in moist peat in glass tubes and kept in an outside insectary at Rothamsted, and the rest, including many damaged, were examined directly or were kept in small batches in the laboratory. These methods enabled the fate of individuals to be watched closely (Table II). Total numbers of flies and parasites were recorded from the whole of the material, but emergence dates (discussed later) were recorded only from that kept in the insectary.

Parasites accounted for between 5.5 (Whittlesey) and 13.7 per cent. (Peterborough) of the deaths observed, and three species, *Aleochara bipustulata* (L.), *A. inconspicua* Aubé (Col., STAPHYLINIDAE) and *Phygadeuon trichops* Thoms. (Hym., ICHNEUMONIDAE) were identified. Parasitisation by *Aleochara* spp. was often evident, but for various reasons, *e.g.*, where only the empty puparium was found or where the parasite died during development, the parasitising species could not be identified.

Taking both Whittlesey and Peterborough material together, about 20 per cent. of the pupae died from various causes other than parasitism. Fifty-one young flies failed to emerge from the puparium or died soon after emerging, and 49 individuals, included as "emerged flies" in Table II, failed to expand and died within a few days. A hundred and sixty-one pupae developed a black pigmentation which affected first the setae, then the appendages and finally the whole body, and 110 became hard and solid without shrivelling or, except for three which also became black, without change of colour. In all, out of 4,802 pupae observed, 3,382, *i.e.*, 70.4 per cent., produced viable flies. It must be remembered that these pupae, unlike the previous ones, were not exposed to predation.

Notes on biology of the parasites.

Aleochara bipustulata.

This species parasitises various Anthomyiid flies (listed by Fuldner, 1960), but there is no published account of its attacking wheat bulb fly. Dr. H. C. Gough, of the National Agricultural Advisory Service informs me (*in litt.*), however, that he has observed it attacking wheat bulb fly on several occasions. Its life-cycle, outlined by de Wilde (1947) and Fuldner (1960) is similar to that of *A. bilineata* Gylh. (Wadsworth, 1915). Eggs are laid in the soil and the active, campodeiform

first-instar larva bores into the host puparium and lives ectoparasitically on the pupa within. There are three larval instars and, at each moult, hypermetamorphosis occurs involving changes in the mandibles, reduction and simplification of the appendages and the assumption of an eruciform condition. The full-grown larva pupates within the empty host puparium and the adult escapes by cutting a slit all round the puparium near one end so that a circular, cap-like portion around its tip is detached. This detached portion is then inverted and transferred to the opposite end before the beetle escapes. Out of 15 empty puparia examined, nine had the emergence hole at the anterior end and six had it at the posterior end.

Adult beetles laid eggs in damp sand in the laboratory and first-instar larvae were seen 12 to 14 days after putting the sexes together. They fed on freshly killed adults of wheat bulb fly, removing the soft tissues and leaving the disarticulated exoskeleton, and attacked eggs, biting them across the middle and extracting the contents. When starved they soon turned cannibal. Adult beetles will also feed on the eggs (Hughes, 1959), on the larvae and on the contents of the broken puparia of *Erioischia brassicae* (Bch.) (de Wilde, 1947).

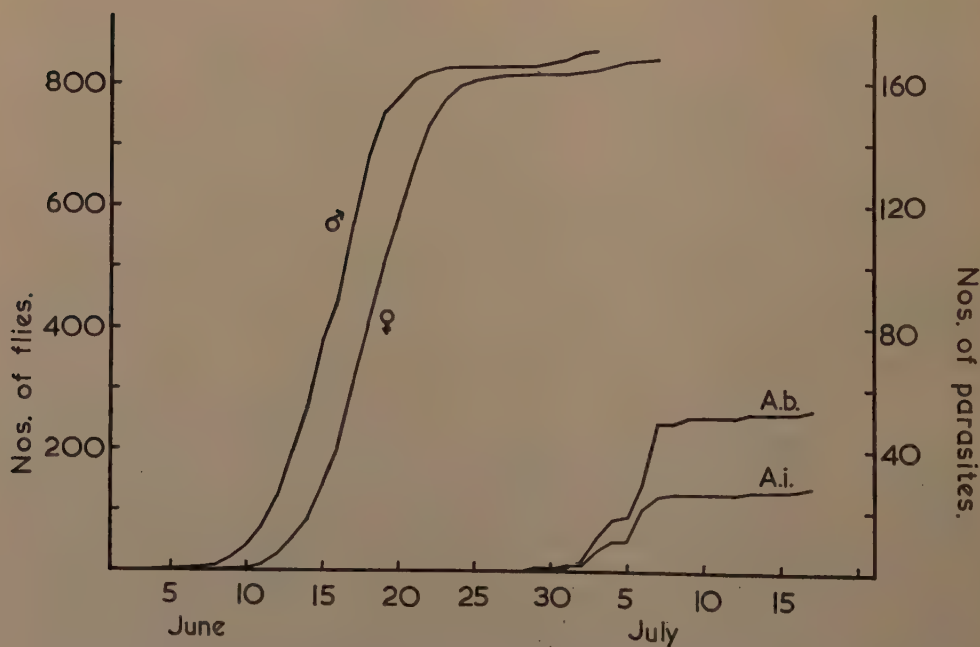


Fig. 1.—Emergence of flies and parasites from pupae collected at Whittlesey in 1959. (♂ & ♀, male and female flies; A.b., *Aleochara bipustulata*; A.i., *Aleochara inconspicua*.)

A. bipustulata was the most abundant parasite found at Whittlesey and Peterborough and, although not in the first batch of pupae from both sites, occurred in all later ones. The emergence data for flies and parasites observed in the Rothamsted insectary (figs. 1, 2) are thought reasonably representative, because the mean emergence dates of flies bred from pupae collected on different dates

were similar (Table III). Had the insectary been warmer or colder than the field, then a trend in the emergence dates of successive batches would have been expected.

TABLE III.

Mean emergence dates of flies obtained from pupae collected at Whittlesey and Peterborough in 1959.

Site	Date of collecting	Mean emergence dates of flies	
		Male	Female
Whittlesey	May 5	June 16	June 19
	12	17	20
	19	16	19
	26	16	19
	June 2	15	19
Peterborough	May 8	June 14	June 18
	15	14	17
	22	13	16
	29	14	16
	June 4	16	18

The adult beetles emerged $2\frac{1}{2}$ to 3 weeks after the flies and, allowing seven to eight weeks from the onset of parasitisation to the appearance of the first beetles (*i.e.*, early May to late June), the entire life-cycle, from pairing of the sexes to appearance of young beetles appears to take 9 to 10 weeks.

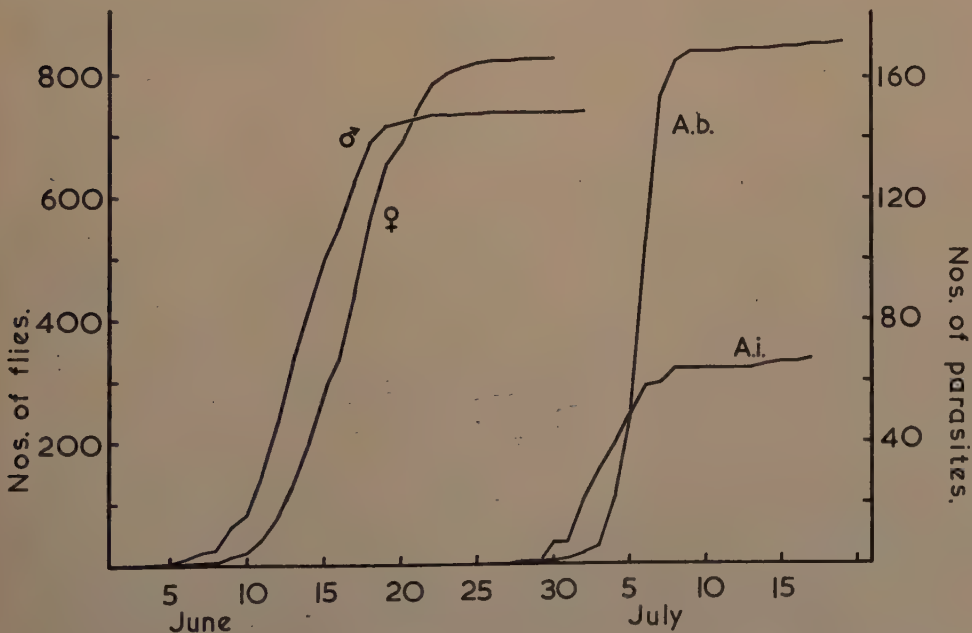


Fig. 2.—Emergence of flies and parasites from pupae collected at Peterborough in 1959.

Aleochara inconspicua.

This species is usually regarded as rare in Britain and was included in Fowler's monograph (1888) as very doubtfully British. Its presence was, however, confirmed by Blair (1933), who found a single specimen near Southwold (Suffolk). Clearly, the species is common at Whittlesey and Peterborough.

There is no published account of its parasitic habit, but it has been obtained from wheat bulb fly previously (C. E. Tottenham, private communication). Its life-history somewhat resembles that of *A. laevigata* Gylh., which also parasitises wheat bulb fly (Speyer, 1954) and of *A. curtula* Goeze (Kemner, 1926) in that the third-instar larva is campodeiform and active, and leaves the host puparium when fully grown. It escapes through a jagged hole in the side of the puparium, usually near the anterior end, and pupates in a small silk-lined chamber in the soil. *A. inconspicua* is a small beetle and its larva consumes only one-half to two-thirds of the contents of the host puparium. Pupation outside its host may therefore be advantageous, for inside there might be risk of being stifled by the decaying remains of the pupa.

As with *A. bipustulata*, the adult beetles fed on the soft parts of eggs and recently killed adults of wheat bulb fly, and they soon turned cannibal when starved. They did not lay eggs in damp sand provided in insectary cultures.

A. inconspicua occurred in all except the last batch of pupae from Whittlesey and was more numerous in the earlier than in the later batches (Table II). (It is believed that its numbers were grossly under-estimated in the first batch from Whittlesey and to a lesser extent in the first from Peterborough because for a time it was not realised that it vacates the host puparium before pupating.) The falling incidence of infestation from batch to batch suggests that many mature larvae had already left the puparia when these were collected and that increasingly higher proportions of stragglers were being obtained. This is supported by a tendency for beetles from the earlier batches to emerge before those from the later ones, *e.g.*, the mean emergence date for those collected at Peterborough on 8th May was 3rd June, whereas that for the remainder of the Peterborough material combined was 7th June. Like *A. curtula* (Kemner, 1926), *A. inconspicua* may attack only young puparia.

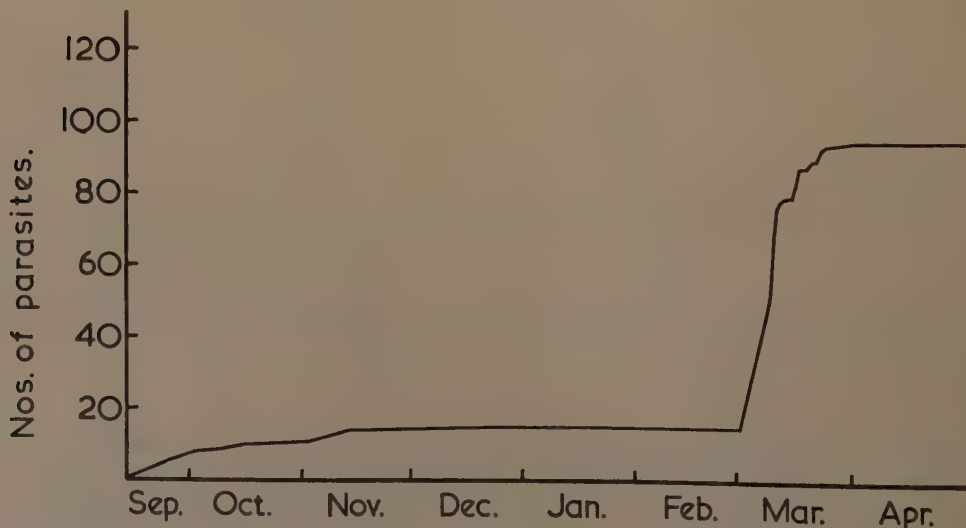


Fig. 3. Emergence of *Trybliographa spaniandra* during 1958-59.

The emergence data (figs. 1, 2) suggest that the beetles emerge $2\frac{1}{2}$ to 3 weeks after the flies, but it must be stressed that these figures may be based on only part of the true population.

Trybliographa spaniandra.

This species was the most abundant parasite obtained from wheat bulb fly at Rothamsted in 1957 and 1958. Females greatly outnumbered males; in 1957, all 81 specimens examined were female and, in 1958, only 2 out of 110 were male. "*Cothonaspis* sp." recorded from wheat bulb fly by Van Miegroet (1950) may have been this species as this name was formerly, but incorrectly, attributed to *Trybliographa* (G. J. Kerrich, *in litt.*).

In 1958, most parasites (18 out of 20 examined) had pupated by August and the first adults appeared in September. The main emergence, however, was not until the next spring (fig. 3). Because of this, it seems likely that, in common with other species of EUCOILINAE (Clausen, 1940), *T. spaniandra* attacks the larva of its host.

Adult parasites survived for some time without food or water (*e.g.*, over 25 per cent. of them survived for four weeks), and although they were quiescent at insectary temperatures, they rapidly became active when warmed.

Phygadeuon trichops.

This species is a well-known parasite of Anthomyiid flies but has not been recorded from wheat bulb fly before. Its biology was studied by Monteith (1956) who showed that the eggs are deposited on the host pupa within the puparium.

Observations on predation of wheat bulb fly.

Reference has already been made to predation by the species of *Aleochara* which parasitise wheat bulb fly. *Scopeuma stercoraria* (L.) (Dipt., CORDYLURIDAE) (the species commonly cited as *Scatophaga stercoraria* (L.)) and *Tetragnatha extensa* (L.) (Araneida) preyed on adult flies in the laboratory, and workers of *Myrmica rubra* (L.) (Hym., FORMICIDAE) removed buried pupae and newly emerged flies from pots of soil in the field.

Simple laboratory tests were devised in which adults of possible predator species, a complete list of which is shown in Table IV, were offered eggs or pupae of wheat bulb fly as food. It is evident from the results in Table IV that *Notiophilus biguttatus*, a species adapted for preying on small moving arthropods (Davies, 1953a), attacked neither eggs nor pupae, but *Clivina fossor* and both the *Bembidion* spp. tested ate eggs readily. *Bembidion* species also eat eggs of *Erioischia brassicae* (Wishart, Doane & Maybee, 1957; Hughes, 1959) and *B. lampros* which is an important predator on the eggs of this species was studied by Mitchell (1958).

The two *Harpalus* spp. and *Bradycellus verbasci* ate eggs, and *H. rufipes*, the only species tested, also ate pupae. Other *Harpalus* spp. tested by Wishart, Doane & Maybee (1956) refused eggs of *E. brassicae*. Davies (1953a) regarded the Harpalini as predominantly herbivorous but as taking animal food in small amounts.

The *Amara* spp., except for *A. aulica*, showed predation, and this is interesting because reports of predation by *Amara* spp. have been few (Allen, 1953; Davies, 1953b) whereas their herbivorous habits are well-known (*e.g.*, Westwood, 1839; Hart, 1884; Webster, 1903; Aubrook, 1949; Davies, 1953a). The attack of *A. familiaris* on the pupa was striking: within minutes of being introduced, the beetle tore open the abdomen of the pupa and consumed much of its contents. A single beetle could destroy several pupae in quick succession.

Feronia melanaria, a pest of strawberries, takes animal food both as a larva (Briggs, 1957) and as an adult (Williams, 1959). It refused eggs of wheat bulb fly

as it refused those of *E. brassicae* (Wishart, Doane & Maybee, 1957). *F. madida*, another pest of strawberries, was not tested with eggs but ate pupae. This species takes both animal and plant food and was regarded as an unspecialised feeder by Davies (1953a).

Agonum dorsale sometimes accepted and sometimes refused eggs, but neither of the individuals tested attacked pupae. *Demetrias atricapillus* attacked eggs but not pupae.

None of the STAPHYLINIDAE tested attacked pupae, but all the *Tachyporus* spp. tested with eggs ate them. Neither *Stenus* spp. nor *Astilbus canaliculatus* ate eggs. The former are specialised for the pursuit and capture of small motile prey (Schmitz, 1943) but will attack maimed adults of Diptera (Vorisi, 1934) and the latter is associated with, and predatory on, ants (Donisthorpe, 1927).

TABLE IV.

Results of laboratory observations on predation of eggs and pupae.

Species tested	Number tested with:		Results	
	Eggs	Pupae	Eggs	Pupae
Coleoptera (CARABIDAE)				
<i>Notiophilus biguttatus</i> (F.)	4	1	—	—
<i>Clivina fossor</i> (L.)	1	0	+	—
<i>Bembidion lampros</i> (Hbst.)	11	0	+	—
<i>B. quadrimaculatum</i> (L.)	1	0	+	—
<i>Harpalus aeneus</i> (F.)	1	0	+	—
<i>H. rufipes</i> (Deg.)	1	3	+	+
<i>Bradycellus verbasci</i> (Duft.)	1	0	+	—
<i>Amara similata</i> (Gylh.)	0	1	—	+
<i>A. familiaris</i> (Duft.)	2	9	+	+
<i>A. aulica</i> (Panz.)	1	0	—	—
<i>Feronia melanaria</i> (Ill.)	1	0	—	—
<i>F. madida</i> (F.)	0	1	—	+
<i>Agonum dorsale</i> (Pontoppidan)	4	2	±	—
<i>Demetrias atricapillus</i> (L.)	1	7	+	—
Coleoptera (STAPHYLINIDAE)				
<i>Stenus clavicornis</i> (Scop.)	2	1	—	—
<i>S. atratulus</i> Erichs.	1	0	—	—
<i>Philonthus fuscipennis</i> (Mannh.)	0	1	—	—
<i>P. varius</i> (Gylh.)	0	1	—	—
<i>Tachyporus chrysomelinus</i> (L.)	1	0	+	—
<i>T. solutus</i> Erichs.	2	0	+	—
<i>T. obtusus</i> (L.)	0	1	—	—
<i>T. hypnorum</i> (F.)	4	1	±	—
<i>Astilbus canaliculatus</i> (F.)	1	0	—	—
<i>Aleochara bipustulata</i> (L.)	Many	0	+	—
<i>A. inconspicua</i> Aubé	Many	0	+	—
Dermaptera				
<i>Forficula auricularia</i> L.	1	0	+	—
Chilopoda				
<i>Lamycetes fulvicornis</i> Meinert	5	0	+	—
<i>Lithobius variegatus</i> Leach	1	0	+	—
<i>Necrophloeophagus longicornis</i> (Leach)	0	2	—	±

+ Predation observed

— No predation

± Variable result

Summary.

Observations in eastern England during 1957, 1958 and 1959 showed natural mortality of pupae of wheat bulb fly, *Leptohylemyia coarctata* (Fall.), to be high. The part of this due to parasitisation is considered in some detail.

At Rothamsted, the main parasite was *Trybliographa spaniandra* Kerrich & Quinlan, and this affected about 10 per cent. of the population in 1957 and about 5 per cent. in 1958. At Whittlesey and Peterborough, in 1959, parasitisation affected 5.5 and 13.7 per cent. of the populations, respectively. The two main parasites found were *Aleochara bipustulata* (L.) and *A. inconspicua* Aubé (Coleoptera, STAPHYLINIDAE). *Phygadeuon trichops* Thoms. (Hymenoptera, ICHNEUMONIDAE) also occurred but was much less frequent. Notes and original observations on the biology of these parasites are given.

Several insects and other small arthropods were observed to prey on various stages of wheat bulb fly. Details of these and notes on the biology of some of them are given.

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AIRCRAFT APPLICATIONS OF INSECTICIDES IN EAST AFRICA.

XII.—PRELIMINARY ATTEMPTS TO REDUCE THE COST OF CONTROLLING THE TSETSE SPECIES *GLOSSINA MORSITANS* WESTW..

G. SWYNNERTONI AUST. AND *G. PALLIDIPE*S AUST. IN
SAVANNAH WOODLAND.

By R. FOSTER,* P. J. WHITE and D. YEO

I.C.

Colonial Pesticides Research Unit, Arusha, Tanganyika.

Although some previous attempts to eradicate savannah tsetse flies (*Glossina* spp.) by insecticides applied by aircraft met with considerable biological success (various authors summarised by Yeo, 1954), they involved the use of relatively large, twin-engined aircraft, and Hocking, Yeo & Anstey (1954) showed that the effective treatment of woodland by this method cost approximately £1,000 per sq. mile. This figure was prohibitive to large-scale work, and, moreover, Hocking (1961) showed that a residual insecticide applied manually to the resting places of *G. morsitans* Westw. in concentration sites of this species reduced the population to a very low level at a cost of under £300 per sq. mile. This paper describes a series of experiments, designed to reduce the cost of aircraft work against tsetse, in which a single-engined Auster J.5G aircraft was used to apply a five per cent. solution of insecticide at a nominal dosage of only 0.08 gal. per acre. The aircraft carried two spraying units, in the form of belt-driven rotary-cage atomisers, which produced a coarse aerosol having a mass median diameter of about 50 microns; its pay-load was 60 gal. of insecticide and it was flown operationally at an indicated air speed of 60–70 miles per hour.

Preliminary small-scale trials (single applications) gave promising results and were followed by a full-scale experiment of seven applications. This failed to eradicate the fly, but formed the basis for the eventual successful operation recorded by Burnett & others (1961).

Small-scale trials.

Four trials were performed, two at a time, in two small isolated blocks of bush infested by *G. swynnertoni* Aust. The second pair was carried out in the same blocks as was the first, after a lapse of time such that emergence from pupae and possibly some immigration had restored the tsetse populations to their original levels. All applications were completed in single sorties immediately after dawn. The swath width was 70 yd., and the beginning of each run was marked by a ground party carrying a sign-board over which the aircraft was flown on a compass course. The length of the runs varied from a half to one mile.

Trial 1.

One block was treated with a five per cent. (w/v) solution of γ BHC in power kerosene at a nominal dosage of 0.08 gal. per acre. Meteorological conditions were very stable, and assuming all old flies caught in the immediate post-treatment period to be survivors, there was a 90 per cent. kill of old males. There is evidence, however, that flies were carried into the treated area by cattle and motor vehicles.

* Present address: Liberian Institute of the American Foundation for Tropical Medicine, Harbel, Liberia, West Africa.

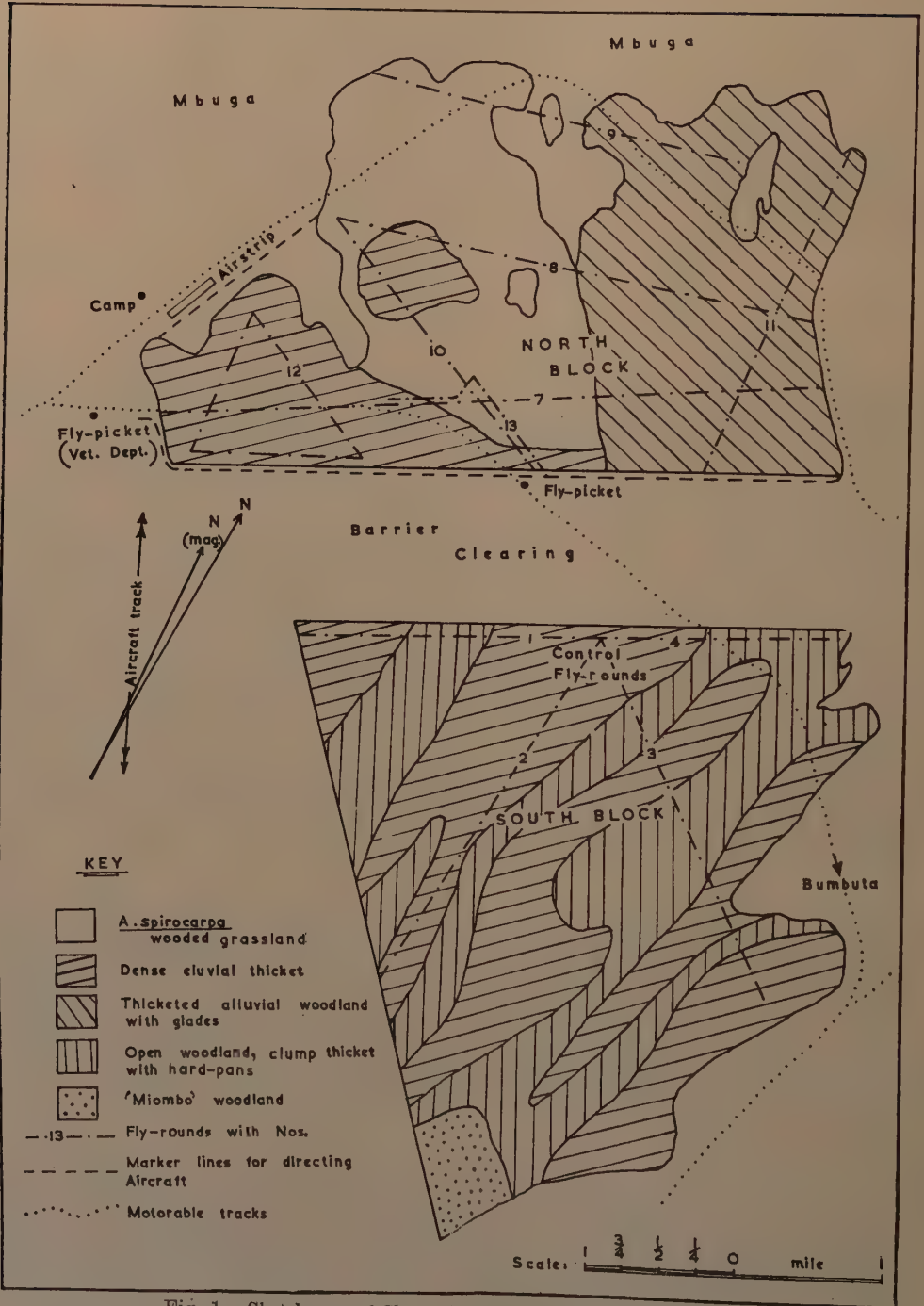


Fig. 1.—Sketch map of Chungai experimental area, 1958-59.

Trial 2.

The other block was treated as described above. Meteorological conditions were stable, and the kill of old males of *G. swynnertoni* was 96–97 per cent. This block also contained an introduced population of *G. morsitans*, and the application effected a 98–99 per cent. reduction among the old males of this species.

Trial 3.

A five per cent. solution of dieldrin was applied at a nominal dosage of 0.08 gal. per acre. Meteorological conditions were again good. There was no immediately dramatic kill as in the treatments with γ BHC, but the population decreased progressively for some days after the application, the final figures indicating a kill of old males of 97–98 per cent.

Trial 4.

An application of γ BHC was made between 0930 and 1030 hr. (East African Standard Time) when meteorological conditions were unstable and rather similar to those met with during the later applications at Chungai (see later). The kill of old males of *G. swynnertoni* was only 90–91 per cent.

Owing to the low numbers caught, it was difficult to assess the kill of old females, but the indicated mortalities were 90 and 95 per cent., respectively, in trials 1 and 2 under good meteorological conditions, but less than 50 per cent. in trial 4 under poor conditions.

The full-scale experiment.*Description of the area.*

General.—The experimental area (fig. 1), known as Chungai (Lat. 04° 39'S., Long. 35° 54'E.), was situated some 20 miles north-east of Kondoia in the Central Province of Tanganyika. Clearing operations in previous years left an isolated area of savannah woodland, several miles from any other known tsetse area, and surrounded by cultivation and open grassland.

This area (25 sq. miles) was too large for the present experiment and was divided into two blocks of about 11 sq. miles each by a mile-wide sheer clearing; the block to the north of the clearing was used as the experimental area, and that to the south served for control catches. Thus the experimental block was bounded on three sides by extensive areas of open grassland, and the only place at which immigration of flies could be expected was across the barrier clearing. This, however, appeared to be an effective barrier, flies penetrating only some 400–500 yd. from either edge. Of 3,586 flies (2,324 of *G. morsitans* and 1,262 of *G. pallidipes* Aust.) marked and released along the southern edge of the clearing during the operational period, only one was recaptured to the north of the clearing. This fly was caught close to the road which passed through the control block across the clearing and into the sprayed area, and which carried considerable foot-traffic. A fly-picket was established on this road before it entered the experimental block, and many flies were caught. Many others must have escaped detection and been carried into the sprayed area, but not in sufficient numbers to account for the experimental results.

Flora and fauna.—The north-eastern part of the experimental block consisted of a thicketed alluvial woodland with glades. The woodland contained *Acacia spirocarpa*, *A. kirkii*, *A. mellifera* and *Lannea humilis*, and the thickets were composed of *Commiphora subsessilifolia*, *C. schimperi*, *Grewia bicolor* and *Albizia anthelminthica*; *Fagara* sp. and *Euphorbia matablense* also occurred. The central area consisted of a more open *Acacia spirocarpa* woodland with little undergrowth, but with a patch of eluvial thicket (described below) around the junction of fly-rounds 8 and 10. The water-holes of the block, mostly seasonal, were

concentrated in this region. The south-western corner of the experimental block consisted of a dense eluvial thicket with small areas of open grassland. The thicket contained *Grewia bicolor*, *G. platyclada*, *Commiphora ugogensis*, *C. schimperi*, *Acacia brevispica* and *Cassia abbreviata*. *Acacia spirocarpa*, *Fagara* sp., *Euphorbia bilocularis* and *Adansonia digitata* also occurred. This eluvial thicket extended as a narrow strip along the southern edge of the block to join the north-eastern alluvial thicket.

TABLE I.

Summary of catches of flies in the experimental block.

Period			<i>G. morsitans</i>				<i>G. pallidipes</i>	
			Old males	Old females	Teneral flies	Apparent density	Total flies	Apparent density
November	1957	..	250	8	8	81	—	—
December	"	..	326	17	10	106	—	—
January	1958	..	326	23	26	106	16	16
February	"	..	333	13	17	108	27	25
March	"	..	288	7	20	94	22	19
April	"	..	306	15	19	99	22	18
May	"	..	168	11	11	55	12	10
June	"	..	157	9	15	51	8	6.9
July 1-9th	"	..	120	8	8	39	11	8.1
First application 9.vii.58—14.vii.58								
1st-2nd application			35	5	11	11	8	3.8
Second application 6.viii.58—11.viii.58								
2nd-3rd application			31	4	10	10	8	3.1
Third application 7.ix.58—14.ix.58								
3rd-4th application			63	15	17	21	24	8.9
Fourth application 3.x.58—10.x.58								
4th-5th application			65	12	21	21	22	12
Fifth application 1.xi.58—8.xi.58								
5th-6th application			74	5	12	24	18	9.7
Sixth application 1.xii.58—8.xii.58								
6th-7th application			55	4	8	18	7	4.0
Seventh application 29.xii.58—2.i.59								
January	1959	..	43	9	7	14	4	2.1
February	"	..	113	6	10	37	8	6.9
March	"	..	140	11	11	46	7	4.7
April	"	..	98	8	8	32	11	7.2

In the control block, the vegetation consisted of alternating north-south strips of eluvial thicket and open woodland clump thicket with hard-pan areas. *Combretum* spp., *Markhamia* spp., *Dalbergia* sp., *Ostryoderris* sp. and *Terminalia* sp. were found in the clumps, with *Dalbergia melanoxylon*, *Commiphora subsessilifolia*, *Acacia mellifera*, *Lannea humilis* and *Euphorbia* sp. in the hard-pans. A small area of 'miombo' woodland occurred at the extreme south corner of the area.

Impala, eland, roan antelope, greater kudu, giraffe, wart-hog, pig and dikdik were resident in the experimental block, and Coke's hartebeest lived near by. Rhinoceros, Grant's gazelle, monkey, elephant, buffalo, lion and zebra visited the area periodically. The fauna of the control area was similar, although elephant and rhinoceros were more common. No large-scale migrations of game were observed.

Distribution of tsetse flies.—*G. morsitans*, although distributed in varying densities throughout the experimental block, was concentrated in the eluvial thicket around the junction of fly-rounds 8 and 10, which area appeared to be the main breeding site of the species. *G. pallidipes* inhabited mainly the south-western thicket, but occurred throughout the area. Three isolated specimens of *G. swynnertoni* were captured.

In the control block, *G. morsitans* and *G. pallidipes* were found throughout, and *G. swynnertoni* was recorded regularly from several localities.

Methods.

A five per cent. (w/v) solution of γ BHC in power kerosene was applied at a nominal dosage of 0.08 gal. per acre (0.04 lb. γ BHC per acre). Seven applications, between July 1958 and January 1959, were made at approximately 28-day intervals.

A marker line was laid out along the edge of the bush (fig. 1), and the start of each run was marked by a ground party as previously described. The aircraft was flown over the marker and the run was continued on a compass course with the aid of a directional gyro and visual reference points. As precession of the directional gyro caused the aircraft tracks to open out, the instrument was periodically re-set during each sortie. The swath width was 70 yd., and most of the runs were about two miles in length; the time taken to complete an application varied from five to eight days. Spraying took place from dawn to about 0800 hr. (E.A.S.T.). Only two evening sorties were possible during the seven months of operations.

Entomological observations.

General.

There were seven fly-rounds in the experimental block (fig. 1). Six were searched for *G. morsitans* and two for *G. pallidipes*; a bait cow was used on all rounds searched for *G. pallidipes*. Searches were made approximately weekly from November 1957 until June 1958, and thereafter about twice per week. During the operation, attempts were made to carry out catches immediately after an application in a particular area, but this was not always possible as no complete fly-round coincided with an area treated on any one day.

Catches of flies in the experimental block are summarised in Table I; apparent densities (*i.e.*, the number of old males caught per 10,000 yd. of fly-path) and monthly totals only are given for *G. pallidipes*, as the numbers caught were too low to warrant detailed analyses for female or teneral flies. All figures are given as monthly means except for the operational period, when data for inter-spraying periods (given in more detail in Foster, White & Yeo, 1959) are amalgamated, and therefore do not estimate kill in any precise manner. Monthly means for flies caught on the control rounds are recorded in Table II; those prior to September 1957 are derived from six fly-rounds in the original entire block before the barrier clearing was constructed, and those for after that date from the four rounds which remained wholly to the south of the clearing after the latter had been completed. Control data for *G. pallidipes* are derived from fly-rounds performed only with screens, and not with bait animals.

Peak densities of *G. morsitans* were recorded in the first quarter of the year (Tables I & II). At the beginning of the long dry season, numbers fell rapidly, reaching a minimum in the period May to June, after which a small secondary peak

was attained in the period September to November. A recession at the end of the year was followed by the rise to peak numbers in the early part of the new year.

Data for *G. pallidipes* suggest a similar pattern. The rise in apparent density from March to June 1956 (Table II) was not subsequently repeated, and after

TABLE II.

Summary of catches of flies in the control block.

Period		<i>G. morsitans</i>			<i>G. pallidipes</i>	
		Apparent density	Old females	Teneral flies	Apparent density	Total flies
March	1956 ..	77	17	24	3.2	19
April	" ..	44	17	9	5.7	27
May	" ..	16	11	11	5.0	31
June	" ..	20	12	15	7.5	29
July	" ..	31	12	12	—	—
August	" ..	34	8	13	—	—
September	" ..	44	18	29	—	—
October	" ..	31	8	9	0.4	5
November	" ..	30	13	18	8.0	33
December	" ..	31	9	11	12	42
January	1957 ..	—	—	—	4.7	27
February	" ..	—	—	—	4.0	22
March	" ..	—	—	—	2.9	15
April	" ..	—	—	—	2.6	12
May	" ..	—	—	—	2.0	10
June	" ..	—	—	—	3.2	10
July	" ..	—	—	—	5.0	14
August	" ..	—	—	—	10	28
September	" ..	—	—	—	17	41
October	" ..	85	4	8	—	—
November	" ..	79	13	6	—	—
December	" ..	119	12	6	—	—
January	1958 ..	119	13	3	—	—
February	" ..	133	13	6	—	—
March	" ..	123	14	7	—	—
April	" ..	102	16	6	—	—
May	" ..	57	12	7	—	—
June	" ..	60	10	9	—	—
July	" ..	59	10	11	—	—
August	" ..	63	14	11	—	—
September	" ..	80	19	17	—	—
October	" ..	85	13	14	—	—
November	" ..	88	19	15	—	—
December	" ..	71	12	12	—	—
January	1959 ..	59	16	13	—	—
February	" ..	70	16	10	—	—
March	" ..	66	16	9	—	—
April	" ..	75	17	12	—	—

reaching a peak in December 1956, the value declined and remained low until August 1957, when a steep rise was recorded. Pre-treatment data for *G. pallidipes* in the experimental block (Table I) show a high density in February, followed by a decline.

There was no real change in the monthly mean hunger stage of the flies between November 1957 and April 1959.

It appears, then, that the reduction in numbers observed before the operation (Table I) is an annual occurrence, and that the population increases annually during the period here covered by roughly the second to fourth applications.

The apparent density immediately prior to treatment suggested that there were (very approximately) 15,000 adults of *G. morsitans* in the experimental area at the time; the population of *G. pallidipes* was possibly considerably higher.

Post-treatment.

The kill of old males of *G. morsitans* in each application is shown in Table III. These figures are derived from an analysis of the original fly-round records from those sectors of each round treated in each individual day of spraying, which are

TABLE III.

The kill of old males of *G. morsitans* in each application.

Application no.	Percentage mortality
1	86
2	89
3	43
4	73
5	47
6	59
7	78

Percentages are based on the mean of the two catches on the appropriate sectors made immediately before an application and all catches made on those sectors within 48 hours of the application.

too bulky for publication but appear in a condensed form in Foster, White & Yeo (1959). Pre-application figures were the means of the two immediate pre-application catches of old males over the appropriate sectors, and post-application figures were derived from all catches made within 48 hours of the spraying of those specific sectors. Some young flies may have emerged and become non-teneral during this period, and the values given in Table III should therefore be regarded as minimal.

After the second application, the apparent density of *G. morsitans* was reduced to approximately ten per cent. of the pre-application value. Immediately prior to the third application the A.D. rose sharply (Foster, White & Yeo, 1959), and although later applications gave differing mortalities, the population generally increased until the end of the year. Four weeks after the last application, the population increased markedly as was usual for the season, and the apparent density in April 1959 in the treated area was roughly half that in the control block, to which, before treatment, it had been almost equal.

It was difficult to assess accurately the kill of old females, but the detailed data suggest that the kill was approximately 50 per cent. in the first application, less in the second and *nil* in the third. On some fly-rounds, the catches increased progressively during and after the third application, post-application figures being among the highest ever recorded. Numbers fell only temporarily after the fourth application, and later applications gave low or negligible kills. During approximately eight months before treatment, old females comprised 4.4 per cent. of the total catch (old males 90.3 per cent.), but between the start of the applications and January 1959 they represented 11.3 per cent. of the catch (old males 71.7 per cent.). After the operation, during March and April 1959, the figures returned to 6.9 per

cent. for old females and 86.2 per cent. for old males. After the first application, the proportion of teneral flies increased because of emergence from pupae unaffected by the insecticide, reaching 41 per cent. of the total catch two weeks after the application, and decreasing as the flies became non-teneral. A similar rise and fall (43 per cent. maximum) was observed after the second application, but after the third application the proportion increased and remained high until after the fifth application, catches after the fourth application being as high as at any time during observations. The mean age of the population of *G. morsitans* (arrived at by the wing-fray method of Jackson, 1946) in the sprayed area fell from 30 days (pre-treatment) to 22 days after the first application and to 14 days after the second application. It remained below 20 days until just before the seventh application, and was consistently lower than that in the control area.

The apparent density of *G. pallidipes* fell markedly after the first application, less so after the second and not at all after the third, when numbers increased generally and remained high until December. Marked decreases followed the last two applications, and the apparent density in February 1959, one month after the end of the operation, approximately equalled the pre-treatment value, but was considerably less than that in February 1958.

Discussion.

The degree of failure of the full-scale experiment was surprising because the dosage of 0.04 lb. of γ BHC per acre was higher than in some previous experiments (for example, Hocking & Yeo, 1956) which gave good control of *G. morsitans*. There was no evidence that flying errors or fly movement between sorties (from unsprayed to sprayed areas) could account for the results.

There were three essential differences in technique in this operation compared with previous ones.

(a) The interval between applications was increased from approximately 14 to 28 days, but previous work (Hocking, Parr, Yeo & Anstey, 1953) suggested that there were no density-dependent factors acting on tsetse populations, and there was no reason to believe that this increased interval would be deleterious; indeed, calculations by Yeo & Simpson (1960) suggested that such an increase would improve the final kill.

(b) In attempting to reproduce previous results at lower cost, some of the operating techniques were pushed as near as possible to the limit, and occasionally the limit may have been exceeded. Particularly, meteorological conditions were far from ideal after the first two applications, but as it was desirable to treat as large an area as possible in a short time, much of the work was performed in

TABLE IV.

The drop spectrum: mean diameter (in microns) of the droplets composing 10, 50 and 90 per cent. (by volume) of the spray.

Installation	Sample of ground deposit			Sample extracted from air by cascade impactor		
	10% of vol.	50% of vol.	90% of vol.	10% of vol.	50% of vol.	90% of vol.
Anson aircraft : exhaust system ..	50 μ	98 μ	170 μ	12 μ	34 μ	80 μ
Anson aircraft : boom and nozzles	43 μ	84 μ	150 μ	18 μ	44 μ	80 μ
Auster aircraft : rotary-cage atomisers	39 μ	61 μ	81 μ	16 μ	44 μ	64 μ

'marginal' conditions, although little was done in really bad weather. Thus insufficient insecticide may have reached the resting sites of the flies, due to atmospheric turbulence or a combination of this and the low volume-dosage. Meteorological conditions, however, could not have been wholly responsible for the results, for they were little different from those during the successful operation of 1959-60 (Burnett & others, 1961).

(c) A smaller aircraft was used and the volume-dosage reduced to 0.08 gal. per acre (0.25 gal. per acre in previous experiments). The drop spectrum, and a comparison with those previously used, is given in Table IV. The principal difference between the present spectrum and previous ones was that it contained fewer large droplets, as shown by the lower mean diameters illustrated in the 90 per cent. columns (Table IV). It was, in effect, a narrower spectrum than those previously used, and if, for example, the rotating cages did not function correctly, then the proportion of droplets in the required size range would fall. During the later applications the rotary atomisers did not always function as planned, and the spray may indeed have contained a high proportion of droplets too large to impact on resting flies. Alternatively, the low volume-dosage and the fact that the drop spectrum was no finer than in previous work may together have resulted in too few droplets of the right size to give an adequate cover of the area. The results of Burnett & others (1961) in the 1959-60 experiment suggest that inadequate cover was probably the main physical reason for the present results.

The basis of the failure was undoubtedly the survival of old females, which was evidenced by the appearance of many teneral flies between September and November. Mean temperatures rose sharply during September, reducing the female pupal period from 54 days (August) to 38 days. The increased emergence rate accounts for the appearance of many teneral flies, but the important point is that those caught during November must have emerged from pupae laid at least after the third application. The increased female percentage during operations also illustrates the survival of females. Subsequent laboratory work by Burnett (1960) has indicated that the lethal dose of insecticide is considerably higher for pregnant females than for other flies, and it now seems probable that many pregnant females received sub-lethal doses during the applications. The unstable meteorological conditions may have contributed to this, for results from the small-scale trial performed under similar atmospheric conditions indicated a very low mortality of old females. Furthermore, Buxton (1955) quotes data suggesting that male flies rest in more exposed positions and that in the early morning they are more active; this would cause a greater amount of insecticide to impact on the males.

The over-all cost of the operation (excluding the construction of the barrier clearing) was approximately £400 per sq. mile, which is less than half of that of previous experiments. This figure, however, is of little significance, as control of the fly was not achieved. Modified techniques were employed in the subsequent experiment described by Burnett & others (1961), which was completed at a cost rather less than in the present operation and achieved a high degree of control. Burnett & others (1961) should be consulted for a discussion of costs.

Summary.

Following successful small-scale trials, an attempt was made, by aircraft application of insecticide, to eradicate *Glossina morsitans* Westw. and *G. pallidipes* Aust. from an isolated block of savannah woodland, approximately 11 sq. miles in extent, at Chungai in the Central Province of Tanganyika between July 1958 and January 1959. A single-engined aircraft, fitted with two belt-driven rotary-cage atomisers, was used to apply a 5 per cent. solution of γ BHC in power kerosene at a nominal dosage of 0.08 gal. per acre (0.04 lb. γ BHC per acre). Seven applications were made at approximately 28-day intervals, the time taken to complete an application varying from five to eight days. The operation failed to control the

flies. Although each of the first two applications reduced the apparent density of *G. morsitans* by about 90 per cent. and that of *G. pallidipes* by a lesser, although still considerable, factor, later applications gave varying and often low mortalities, and the populations increased slowly for some time, the insecticide applications causing only temporary depressions in numbers. Numbers fell towards the end of the operation, but final reductions were only about 50 per cent. or less. Kills of female flies were low, and this undoubtedly led to the eventual failure.

The low volume-dosage, a drop spectrum that possibly contained too few droplets of the required size, meteorological conditions, and biological factors that apparently favoured the survival of female flies are suggested as contributory elements to the low mortalities.

Operational costs were considerably lower than in previous work.

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AIRCRAFT APPLICATIONS OF INSECTICIDES IN EAST AFRICA.
 XIII.—AN ECONOMICAL METHOD FOR THE CONTROL OF
GLOSSINA MORSITANS WESTW.

By G. F. BURNETT, D. YEO, A. W. D. MILLER and P. J. WHITE

Colonial Pesticides Research Unit, Arusha, Tanganyika.

The North Block, Chungai, in the Central Province of Tanganyika (04° 39' S., 35° 54' E.) used to support populations of *Glossina morsitans* Westw. and *G. pallidipes* Aust. The block was treated with a solution of γ BHC in power kerosene by an aircraft in 1958 but, although the experiment gave much information, the fly was reduced by less than 50 per cent. The experiment is described in detail in the preceding paper (Foster, White & Yeo, 1961). For the experiment described here, various modifications in procedure were made, and the use of the same block in two successive years gives unusual possibilities for direct comparisons.

Methods.

The insecticide used was a solution of dieldrin in oil. A concentrate containing 20 per cent. dieldrin in a special high aromatic solvent was diluted before use with Shell power kerosene to give a solution containing 2.5 per cent. dieldrin. This solution was applied at a nominal expenditure of 0.125 gal. per acre eight times with a planned interval of 28 days (although this was varied in practice by events beyond our control). The same aircraft and equipment were used as in 1958: an Auster J5G carrying two Britten-Norman 4-in. rotary-cage units (model A-100, belt drive) which produced a coarse aerosol of volume median diameter 50–60 μ . The cages were modified for the last two applications by lining them with four layers of glass-fibre tissue. A somewhat hurried assessment showed a reduction in large drops by the use of this modification, but this work requires repeating. The swath width was 55 yd., and an overlap of only one run was allowed between consecutive days. This limitation was imposed by the small daily progression made by a single small aircraft over a large block. For the first three applications, all runs were made over a single marker which moved along the southern edge of the block, but for the last five a second marker moved along the northern edge of the block and the aircraft flew alternately in each direction (see fig. 1). This cut non-productive flying to a minimum and made possible complete coverage in four days when favoured by the weather, instead of five or six. Runs were made on the magnetic compass and tracking was exceedingly accurate. The aircraft flew from a bush strip constructed close to the block. Most work was done immediately after dawn.

Description of area.

The block (fig. 1) is described in detail in the preceding paper, which includes a vegetation map. It is about 11 sq. miles in area, separated from the South Block by a barrier clearing one mile wide. By mid-1959, regeneration had been prolific in the clearing, but there was no top cover, and the growth was thick and tangled. There had been a good deal of settlement in the clearing, and paths and gardens may have provided better facilities for tsetse to cross than appeared likely on first inspection. There had been scattered settlement in the experimental block as well, but it was not yet dense enough to disturb fly except on part of fly-round 12 (see fig. 1). Game was probably less plentiful than in the previous year,

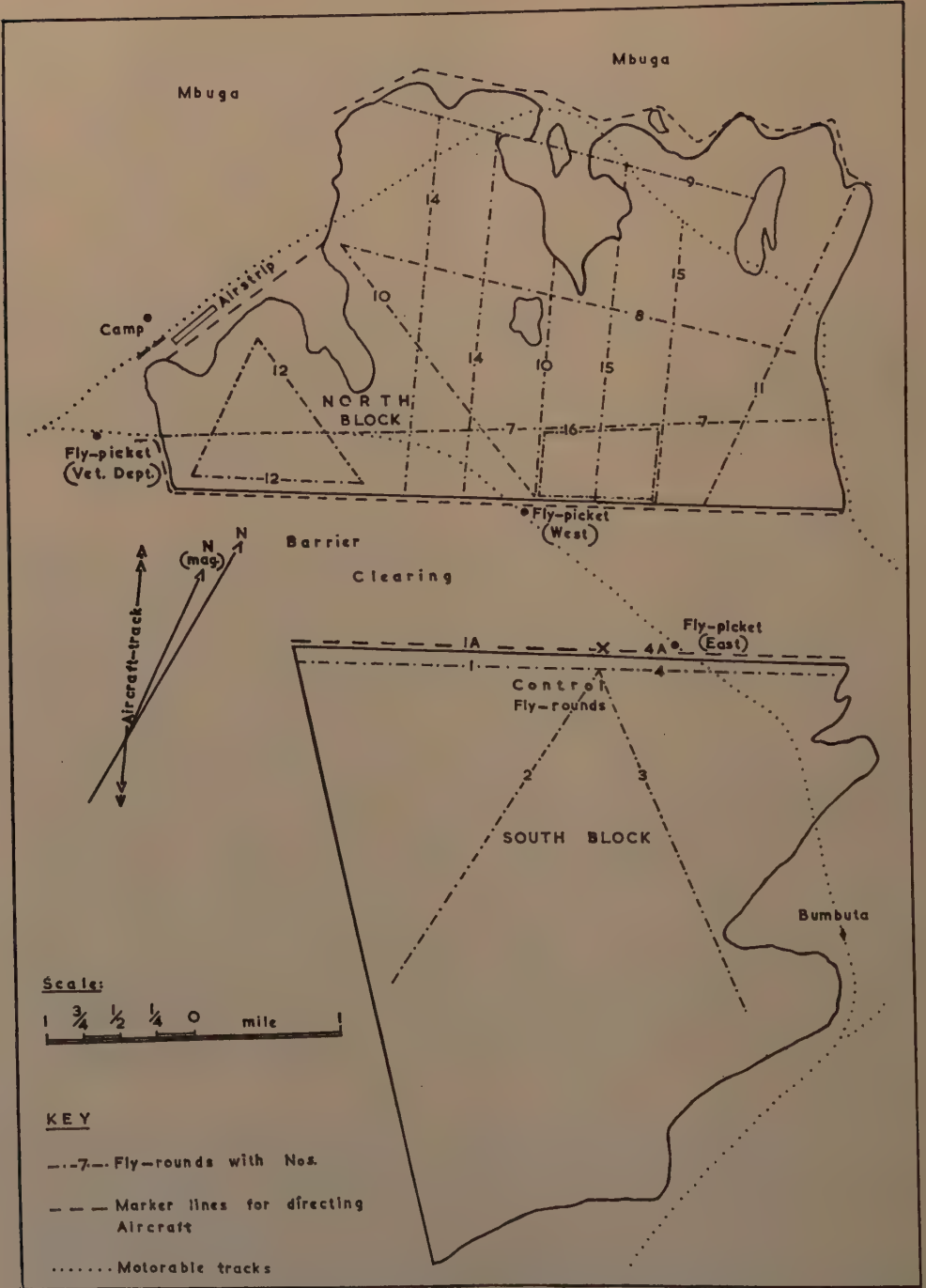


Fig. 1.—Sketch map of Chungai experimental area, 1959-60.

and although impala and roan were always resident in the block, rhinoceros and elephant were not seen (although their spoor was found). Increasing settlement in the whole vicinity may have been responsible for this.

Fly-rounds.

The seven rounds of the previous year were not well laid out for following the course of an insecticidal application, and two extra ones (14 and 15, see fig. 1) were added. This brought the total length of round traversed to assess the population of *G. morsitans* to 30 miles. An additional round (16) was laid out in the habitat of *G. pallidipes*, and two rounds, 12 and 16, of total length 5 miles, were utilised for bait-cattle rounds to collect this species.

Control figures were provided by catches in the South Block made by fly-boys of the Tanganyika Veterinary Department. They are from the same rounds as those given by Foster, White & Yeo (1961, Table II) and may be converted to apparent densities by multiplying by 0.67. In addition to the picket on the road where it left the north edge of the clearing, in December 1959 another was set up at the south edge, catching off people as they entered the clearing. These pickets are shown on the map as fly-picket (west) and fly-picket (east), respectively. In March 1960, the east picket was instructed to catch off eastbound travellers as well. Between April and September 1960, males and females of *G. morsitans* were caught, marked with paint and released in the South Block on the far side of the clearing in an attempt to detect migration across it. Marking was carried out on paths 1, 1A, 4 and 4A; the colour code used distinguished between dates and paths.

Results.

Operational observations.

The experiment started a month late for reasons beyond our control. The fourth application was cut short by unserviceability of the aircraft when about one-sixth of the block (the eastern edge) remained to be covered. The fifth application was brought forward ten days in an attempt to offset this omission, but the start of the seventh was delayed by waterlogging of the airstrip until 48 days after the sixth had started. It was also curtailed and a small part of the eastern end was left unsprayed. The eighth and last application was carried out between rainy periods, and only about two-thirds of the block could be covered before the airstrip was flooded. However, the central part of the block with the principal fly foci was treated.

Emission of spray when flying in both directions reduced non-productive flying by 45 per cent. and permitted more ground to be covered during the limited period of stable conditions after dawn. Meteorological conditions varied. For the first two applications they were good. The third and fourth applications were done in conditions so bad that spraying was continued only because it was highly unlikely that they would improve either the same day or the next. Even at dawn, winds were relatively high, 6–8 ft. per second. The last four applications were made in good to very good conditions. Altogether, evening sorties were possible on nine days and, after afternoon rain, conditions were often better than in the morning.

The flying problems encountered in this experiment were the same as in the previous year but they were dealt with more effectively. Steering was done on the magnetic compass instead of the directional gyro and tracking was very much improved, suggesting that the gyro was subject to uneven precessional errors which went unnoticed the previous year. By working from both ends of the block, coverage was greatly improved because the two marker lines provided a double check on tracking for the pilot and errors were consequently reduced considerably and prevented from accumulating.

TABLE I.
Catches of isetse flies at Chungai.

Period	<i>G. morsitans</i>					<i>G. pallidipes</i> Total flies	
	North Block				Control	N. Block	Control
	Non- teneral males	Non-teneral females		Teneral flies	Non- teneral males		
		Catch	%				
May 1959	177	11	5.9	10			
June 1959	128	6	4.5	5	130	12	3.5
Week ending							
July 4	142	9	6.4	4	130	9	3
11	135	12	8.2	10	112	10	0
18	178	16	8.3	10	127	22	4
First application 22.vii—28.vii.59							
Aug. 1	7	3	30	5	150	2	7
8	2	1	33	22	115	4	1
15	3	2	67	31	189	6	3
22	63	8	11	8	144	4	0
Second application 17.viii—22.viii.59							
Sept. 29	4	2	33	4	157	3	3
5	5	6	55	34	245	10	5
12	20	4	17	14	161	4	6
19	36	7	16	7	122	10	4
26	67	9	12	3	122	—	14
Third application 22.ix—29.ix.59							
Oct. 3	4	1	20	0	140	1	1
10	5	1	17	4	147	0	9
17	3	6	67	7	175	0	1
24	2	4	67	4	222	1	7
31	13	5	28	3	226	0	10
Fourth application (incomplete) 27.x—31.x.59							
Nov. 7	2	0	0	1	242	1	
11	6	2	25	2	225	2	
Fifth application 14.xi—18.xi.59							
Dec. 21	1	0	0	1	221	0	
28	1	1	50	4	169	1	
5	5	1	17	4	157	1	
12	9	1	10	1	170	1	
Sixth application 15.xii—18.xii.59							
1960							
19	0	0	—	0	140	—	
26	0	0	—	0	162	—	
Jan. 2	1	1	50	0	185	1	
9	2	1	33	1	158	2	
12	1	0	0	1	226	0.5	
23	incomplete				88	0.5	
30	4	1	20	0	137	0	

TABLE I.—*cont.*

Period	<i>G. morsitans</i>					<i>G. pallidipes</i> Total flies	
	North Block				Control	N. Block	Control
	Non-teneral males	Non-teneral females		Teneral flies	Non-teneral males		
		Catch	%				
Week ending	Seventh application (incomplete) 1.ii—4.ii.60						
Feb. 6	0	0	—	0	174	0	
13	0	0	—	0	182	0	
20	0	0	—	0	212	0	
27	0	0	—	0	191	0	
Mar. 5	0	1	100	0	141	0	1
12	1	0	0	0	113	0	3
	Eighth application (incomplete) 12.iii—14.iii.60						
19	0	0	—	0	135	—	2
26	0	0	—	0	156	0	3
April ..	0.6	0	0	0	169	0	7.7
May ..	0.95	0	0	0	237	0	8.2
June ..	1.9	0	0	0	238	1	21
July ..	0.75	0	0	0	262	0	16
Aug. ..	1.50	0	0	0	237	—	24
Sept. ..	1.50	0.5	25	0.15	189	—	7.3

The further experience gained with the spray gear since 1958 enabled a better watch to be kept for partial functional failures such as occurred in that year, and it is believed that the gear worked to a consistently high standard throughout the eight applications. The question of providing a marker that can be seen two or three miles away by the pilot is still to be solved.

Entomological observations.

In Table I are given the catches on all fly-rounds. Because a complete set of rounds was done for each entry except in April 1960, the actual catches are given. They can be converted to apparent densities by multiplying catches of *G. morsitans* by 0.2 and of *G. pallidipes* by 0.9. In April 1960, rounds 9 and 11 (see fig. 1) were not searched because of difficulty of access, but they lie in an area which never had many flies (8 were caught in the previous 38 sets of rounds), and their omission has been ignored. The figures for May and June 1959, and for April 1960, are means per set of rounds, but from July 1959 to March 1960 the catch for each set of rounds is given separately. From May 1960, all rounds were covered at least once a month, but rounds 7, 10, 12 and 14 were covered an extra four or five times in an attempt to capture marked flies that might have immigrated. Thus the part of the block which supported most flies before the experiment was covered several times in the month. The monthly catch for the block as a whole during this period is calculated by summing the mean catch for each round, however many times this was covered in the month.

The mortalities achieved at each application are listed in Table II. These are calculated from immediate pre- and post-spraying catches on the fly-rounds; because the latter did not always fall in a single calendar week there are discrepancies between Table II and Table I, in which catches are given by calendar weeks. The pre-spraying catches are too small for these mortalities to be more

than approximate except in the case of males for the first three applications. There is some evidence that flies took several days to die; this has been confirmed in the laboratory (Burnett, 1960) and was noted at Loljoro by Foster, White & Yeo (1961). It is shown by the crude figures for the first application, and although the decrease in the week ending 8th August is due in part to the extended period taken by the application (some of the flies caught in the previous week had not been exposed to insecticide), some fly-rounds when considered alone show this feature. Details are given in a report (Burnett & others, 1960).

TABLE II.

Recorded mortalities of *G. morsitans*.

Application	Kill (%)	
	Males	Females
1 ..	99	94
2 ..	94	87
3 ..	94	89
4 ..	85	100
5 ..	87	100
6 ..	100	100
7 ..	100	100
8 ..	100	—

For method of calculation see text.

Settlement.

Large-scale settlement of the block by settlers accompanied by their domestic stock took place very rapidly and we were informed that by September 1960 there were estimated to be 500 cattle within and near the edge of the block. If this continues, settlement should finally exterminate any fly remaining. The South Block has not been settled.

Discussion.

Immigration.

The population surviving an experiment of this nature can only be properly assessed after pupal production and emergence balance and the age structure is normal. If very few insects survive, it may take several months to collect enough data for a valid comparison with the pre-treatment population, and, if immigration is taking place, the conclusion may be falsified to some extent because the effects of immigration will be much more apparent with a small residual population than they were with the high initial numbers. In the present case, immigration was almost certainly taking place (see below) but it was not on a large enough scale relative to the block as a whole to cause any sustained increase in catches, although control catches of both species rose rapidly after the last insecticidal application. An attempt was made to measure immigration by marking flies on the far side of the barrier clearing. A total of 4,236 males and 459 females was marked between May and September 1960, 300 and 159, respectively, on paths 4 and 4A, which were crossed by the road (fig. 1), and the rest on the other two paths. Of these marked flies, none was recaptured free in the North Block and only 39 at the pickets. At the west picket, 20 were caught, all marked on paths 1 & 1A: at the east picket, 19 were caught, 3 marked on paths 4 and 4A and 16 on 1 & 1A. The higher proportion of flies caught at the west picket that had originated at the far

end of the block from the road suggests that they might have crossed the clearing direct and not *via* the road. However, the difference in the constitution of the catch at the two pickets is not significant (P exceeds 0.1). The quite considerable numbers of flies caught at the pickets are given in Table III, and, since no picket is flyproof, many flies must have evaded them and entered the block. If these

TABLE III.

Catches at fly-pickets; sexes combined.

Picket	<i>G. morsitans</i>			<i>G. pallidipes</i>		
	West	East (west-bound)	East (east-bound)	West	East (west-bound)	East (east-bound)
Period						
July 1959 ..	183			Included in totals of <i>G. morsitans</i>		
Aug. ..	139					
Sept. ..	211					
Oct. ..	260					
Nov. ..	245					
Dec. ..	141	158				
Jan. 1960 ..	95	144				
Feb. ..	100	123		2	9	
Mar. ..	90	151	52	0	1	1
April ..	64	62	50	0	0	0
May ..	37	90	35	2	4	3
June ..	76	146	60	39	52	10
July ..	95	157	66	11	51	9
Aug. ..	180	315	106	25	36	19
Sept. ..	170	69	205	11	7	14

The east picket started to catch off eastbound traffic on 15th March 1960.

flies had come across the clearing and been picked up along the road, it would suggest that many others were crossing direct along the whole length of the clearing. When the east picket was set up, there was a considerable drop in catches at the west picket, and so we may conclude that a proportion at least originated in that part of the south block traversed by the road. There was a further drop when catching off eastbound traffic started at the east picket. It is thought that this was because a number of flies hung about the road, avoiding the pickets more or less successfully, and that the extra catching reduced the number of flies available for traffic to pick up on its westward journey. It is obviously impossible to draw firm conclusions from the evidence on the extent of immigration, but quite evident that some took place.

After completion of the treatment, no females were caught in the block until September 1960, when four were captured. Two had been fertilised and two were virgin. The first teneral fly was also caught in September.

No examples of *G. pallidipes* were marked, but it is a notoriously far-ranging tsetse and the great increase in catches in the control block and at the pickets in mid-1960 (Tables I & III) make it probable that it was spreading widely. This was confirmed by a number of catches on the rounds patrolled for *G. morsitans*—a total of 18 from May to September, equal to the number of old males of *G. morsitans*—and the presence of this species in places outside the South Block where it had not been met with for several years (Mr. J. Howard, personal communication).

Residual population.

Catches made during the first six weeks (about $1\frac{1}{2}$ pupal periods) after the last application have been omitted from consideration and catches over the following three months and four months, respectively, have been pooled for the purpose of calculating the residual populations of *G. pallidipes* and *G. morsitans*, respectively. Catches for these periods have been compared with those for the eight weeks of June and July 1959 before the first application. During both these periods, catches were stable, but in the interval between them control catches of *G. morsitans* (non-teneral males) increased 1.9 times and of *G. pallidipes* (total flies) 5 times. If post-treatment catches are reduced to allow for this, the percentage reduction in both becomes 99.5 per cent. These results may be compared with the theoretical treatment due to Yeo & Simpson (1960, Table II) who show that a repeated kill of 65 per cent. should give such a reduction. The recorded kill of *G. morsitans* per application (Table II) was 85 per cent. or above, numbers of *G. pallidipes* being too small for mortalities to be calculated but these were probably not much less. This sort of discrepancy has been noticed several times in earlier aerial experiments (*e.g.*, Hocking & others, 1953 (South Block); Hocking, Burnett & Sell, 1954*a*, *b*) but not, so far as is known, when using residual treatments. It is believed that laboratory work in progress explains this inconsistency. It has been found that, when chlorinated hydrocarbon insecticides are applied topically in oil solutions, old female flies, especially when pregnant, have a very much greater tolerance than young males or young females. (Burnett, The effect of age and pregnancy in tsetse flies in increasing tolerance to insecticides (in press).*) The mean life of wild males is 2-4 weeks (Jackson, 1949), therefore most wild males are young and susceptible to insecticide. Female flies come to man most readily in the first three weeks of adult life (Jackson, 1946, 1948), that is, when they are susceptible to insecticide. The older, reproductively active section of the female population is not readily available and a high kill among the young flies of both sexes may be accompanied by a low mortality of old females; but this will not be detected by the usual survey methods. However, the extinction of the tsetse population as a whole depends on the extermination of that part which is active in reproduction and, if this suffers only a light mortality, extinction will be reached only slowly. It is no doubt significant that the female percentage, but not the number caught, rises abruptly after each application and then falls as young flies emerge and mature (Table I). In most of our earlier trials it was notable that the female percentage was high in the few captures made during the course of a series of applications (*e.g.*, Hocking & others, 1953; Hocking, Burnett & Sell, 1954*a*; Hocking, Yeo & Anstey, 1954). In these papers only non-teneral males and total flies are tabulated, but examination of the original data shows that most of the difference is non-teneral females.

Comparison with previous experiment.

The two experiments made on this block should be compared. Relevant data are summarised in Table IV. When the final reduction in numbers is calculated on a similar basis for both experiments, by waiting for the post-treatment population to become stabilised, as explained above, the reduction achieved in 1958 was 38 per cent. for *G. morsitans* and only 12 per cent. for *G. pallidipes*. There is, however, no control figure by which the latter can be corrected. The measured kill for each application was almost always higher in 1959, especially for the later applications (compare Table II with Table III in Foster, White & Yeo, 1961). It is therefore likely that the difference in final result lies in technique rather than accidents of meteorology or operational procedure—the extra application made in the second experiment could merely increase the final reduction in numbers of

* To appear shortly in *Nature*, *London*.

tsetse from 99.02 to 99.5 per cent. (Yeo & Simpson, 1960) and it was in any case incomplete. Certain differences in technique were planned, *e.g.*, the reduction of the swath width, increase in volume dosage and the change of insecticide and halving its concentration. Topical applications of dieldrin and γ BHC have shown that the relative potency is about 4:1 for young males (95% fiducial limits 2.7-5.6) and 3.6:1 for young females (fiducial limits 2.5-4.9) (Burnett, The susceptibility

TABLE IV.

Comparison of the two experiments carried out in the North Block, Chungai.

	1958	1959-60
Insecticide .. \	γ BHC	Dieldrin
Concentration (%)	5	2.5
Swath width (yd.)	70	55
Volume dosage (gal./acre)08	.125
Dosage (lb./acre)04	.031
Number of applications	7	8
Number of applications not completed	0	3
Interval between applications (days)	28	28
Size of drop lethal to 95% of young males	55 μ	40 μ
Size when emitted	85 μ	75 μ
Approx. relative volume of spray per acre dispersed as drops lethal to 95% of young males	1	3
Reduction in catches (%)		
Non-teneral males of <i>G. morsitans</i>	38*	99.5**
<i>G. pallidipes</i> , total catch	12*	99.5***
Theoretical kill (%) per application (Yeo & Simpson, 1960), both species	much less than 50	65

* Calculated from means of 9 weeks before and weeks 5-18 following applications.

** Calculated from means of 8 weeks before and weeks 7-23 following applications.

*** Calculated from means of 8 weeks before and weeks 7-19 following applications.

Adjusted for control catches where available.

of tsetse flies to topical applications of insecticides. I.—Young adults of *Glossina morsitans* Westw. and chlorinated hydrocarbons*). Although old females are much less susceptible than young flies of either sex (Burnett, see footnote on p. 312) they are still much more susceptible to dieldrin than to γ BHC, and 2.5 per cent. dieldrin remains more toxic than 5 per cent. γ BHC (unpublished data). Moreover, a lethal dose is contained in a smaller droplet of insecticide and because the proportion of volatile solvent is greater the difference is increased by evaporation. The LD95 for young males is contained in droplets of 75 μ (dieldrin) and 85 μ (γ BHC) when formed, but in a few seconds these are reduced to 40 μ and 55 μ . (The ratio of LD95's is lower than of LD50's although the lines are statistically parallel at $P=0.05$, and the comparison has been made using LD95's in order

* To appear in a later part of *Bull. ent. Res.* 52.

to favour γ BHC). Droplets below 50μ in diameter are considered better able to penetrate vegetation and impact on tsetse than larger droplets. Thus the change appears to have been a considerable improvement. Consideration of the drop spectrum used (Foster, White & Yeo, 1959, fig. 2, and 1961, Table IV) enables us to calculate that for young males the potential volume of lethal drops per unit area in 1959-60 was three times that of 1958 (Burnett, 1960). It is not yet possible to make similar calculations for breeding females.

Other favourable influences such as reduced swath width, improved flying techniques and more reliable dispensing equipment combined to provide more even coverage, always one of the principal difficulties in aircraft work (see Burnett & Thompson, 1956). It is impossible to disentangle from the complex of factors the effect of any particular one, but it is clear that success was only barely attained and there is no scope for modifying the technique, to cheapen it, in any way which reduces its efficiency.

Costs.

Emission of spray when flying from both sides of the block made economies in non-productive flying time, and in fact there is very little prospect indeed of reducing flying costs further. To compare the two experiments, the costs have been calculated on identical bases; it is assumed that the second experiment was conducted in the modified way (see p. 305) from the beginning and that eight complete applications were carried out. Costs have been divided into two heads: (a) on the spot—flying, pilot's salary, ground party; and (b) those due to the particular locality of the experiment—transport of insecticide and personnel by road, and transit flying. Aircraft depreciation is not included.

	a				b
	Insecticide	Flying, etc.	Total	Per sq. mile	Per sq. mile
1958	£2,485	£1,080	£3,565	£325	£55
1959-60	£2,563	£741	£3,304	£301	£66

When comparing these two it must be borne in mind that the earlier experiment failed to reduce fly sufficiently for large-scale settlement with stock to take place while the second succeeded in doing so. Without delays and incompleting applications (and with no possibility of reinfestation) an unequivocal result might be expected and the fly exterminated. In any event, this was the cheapest aerial operation mounted against tsetse and, if judged by the speed at which settlement with cattle has taken place, one of the most successful.

The future of aerial work against tsetse flies, except for special circumstances (*e.g.*, Hocking & Yeo, 1956), depends on cheapening the operation because it is necessary to outweigh the disadvantages of the need for isolation of treated areas and the need to operate on a large scale, and so spend a lot of money in a short time, if one is to operate at all. The operation here described succeeded, but there was little or no margin, and there is no scope for economies in insecticide or flying unless they are compensated for by corresponding increases in efficiency. There are, however, two obvious ways of reducing costs. First, the use of a more lethal, but no more costly, insecticide, or at least one where extra lethality more than outweighs extra cost. This may not be easy to find, for tsetse are exceptionally susceptible to dieldrin, and the cost of the diluted solution is largely determined by that of the diluent, but there is an additional advantage in a more lethal insecticide—a lethal dose may be carried by a smaller drop, better able to penetrate

vegetation. Secondly, costs may be reduced by the use of disseminating equipment which produces no large drops. A single large drop will produce eight of half the diameter, and, if each of these carries a lethal dose, the gain in insect/insecticide contact is considerable. Depending on the exact drop-size distribution and the size of a drop containing a lethal dose, the proportionate increase in useful drop numbers may be calculated and volume dosage reduced an equivalent amount. This would be a direct saving, but, in addition, each aircraft load could cover a larger area, thus taking full advantage of the usually short period of good meteorological conditions and at the same time reducing the proportion of transit flying from base to block.

Summary.

In the North Block at Chungai, comprising about 11 sq. miles of thorn savannah and thicket in Central Province, Tanganyika, an Auster J5G aircraft was used between July 1959 and March 1960 to apply a 2.5 per cent. solution of dieldrin in oil at the rate of 0.125 gal. per acre in an attempt to eradicate *Glossina morsitans* Westw. and *G. pallidipes* Aust. Eight applications were made at approximately four-weekly intervals. Swath width was 55 yd., and the aircraft emitted the insecticide as a coarse aerosol of volume median diameter 50–60 μ as it flew in both directions over the block.

The operation suffered delays, and three applications were incomplete to varying degrees. Kills of *G. morsitans* per application appeared to be 85 per cent. or higher, but the final reduction of 99.5 per cent. could theoretically have been attained with consecutive mortalities of only 65 per cent. It is suggested that this discrepancy may be due to the higher lethal dose required by pregnant females. *G. pallidipes* was also reduced by 99.5 per cent. Numbers of fly were reduced sufficiently for large-scale settlement with cattle, which should complete the work of exterminating the fly.

The experiment is compared with that of the previous year in the same block, using γ BHC, which reduced fly catches by less than 50 per cent. It is concluded that a combination of reduced swath width, greater volume dosage, more lethal insecticide and smaller lethal drop, together with improved flying technique and the more reliable performance of the disseminating equipment, was responsible for the improved result. It is thought that without delays, interruptions, incomplete applications and reinfestation, even better results would be obtained.

This was the cheapest and one of the most successful aerial operations carried out against savannah tsetse. Costs actually over the ground were £301 per sq. mile; incidental costs due to the locality of operations were £66 per sq. mile. There is little chance of reducing the costs of flying directly, but economies are possible by the use of other insecticides or, more probably, by more efficient dispensing equipment.

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STUDIES ON THE BITING HABITS AND MEDICAL IMPORTANCE OF
EAST AFRICAN MOSQUITOS IN THE GENUS *Aedes*. II.—SUBGENERA
MUCIDUS, *DICEROMYIA*, *FINLAYA* AND *STEGOMYIA*.

By A. J. HADDOW

The East African Virus Research Institute, Entebbe, Uganda.

The various series of catches on which the present communication is based have been described in the preceding paper in this series (Haddow, 1960) to which reference should be made for detail. For the moment it is enough to note that all were 24-hour baited catches carried out by methods described elsewhere (Haddow, 1954) and that while some were confined to a single level others were conducted at as many as five levels simultaneously, the highest station involved being at 82 feet, in a semi-emergent tree in heavy rain-forest. The total number of catches involved is 448. All were carried out in East Africa, and 401 were made in forest, in Uganda. Catch time is so adjusted that 1800 hr. always corresponds to the time of sunset (Lumsden, 1952) and, as all the work was carried out very close to the equator, sunrise may be taken, for practical purposes, as 0555 hr.

In the preceding paper, the subgenera *Aëdimorphus*, *Banksinella* (now *Neomelaniconion*) and *Dunnius* were discussed. These mosquitos are, with few exceptions, ground-haunting species, and the description of their circadian* biting rhythms is comparatively simple. Many species in the other subgenera of *Aedes*, however, show arboreal tendencies of varying degree and, while each species shows, as a rule, a preference for some particular level, many appear to make daily vertical migrations. Thus biting may reach its maximum intensity at different times at ground-level, in the understorey and in the canopy, and in such cases each level must be considered separately. A second complication is introduced by the fact that, while some species show similar behaviour throughout their geographical range, others vary in habits from one area to another. Such a case has already been described in the case of the genus *Eretmapodites* (Haddow, 1956) and others will be discussed below. Thus, in many instances, where complex biting behaviour is to be discussed, each area also must be taken separately. Often the most satisfactory method, where biting cycles are concerned, is to discuss in detail a representative series of catches from a productive area, subsequently noting such differences as may have been observed elsewhere.

A further point of importance that has begun to emerge during study of the large mass of data now available is that under certain circumstances a given species may fail to show a clear pattern of biting behaviour, no matter how long the investigation is continued. A nocturnal species, for example, will probably (though not necessarily) retain its nocturnal character under such conditions, but may show no clearer periodicity than this whereas, under favourable circumstances, it may show a definite and characteristic peak of activity at some particular time during the night. At the moment it appears that such loss of biting pattern may be brought about by a number of factors, among which the following are believed to be important:

- (1) *The use of a bait not normally attacked by the mosquito concerned.* A good example of the type of almost patternless behaviour encountered in such circumstances is given by the figures quoted in the preceding paper in this

*The term 'circadian' denotes a daily period that may differ from 24 hours by not more than a few hours (see Halberg & others, 1960).

series for the small, silver-spotted species of *Aëdimorphus* of the groups of *Aëdes apicoannulatus* (Edw.), *A. argenteopunctatus* (Theo.) and *A. domesticus* (Theo.) (as defined by Edwards, 1941), which probably do not bite man or other primates except casually. Much the same is to be seen in non-man-biting genera such as *Uranotaenia* and *Ficalbia* and also in many species of *Culex* and some *Anopheles*. The rule has of course exceptions, for example, those species of *Mansonia* which have been shown to feed almost exclusively on birds (Williams, Weitz & McClelland, 1958; McClelland & Weitz, 1960) but which exhibit a clear and well-defined biting pattern when attacking man. In most cases, however, it seems to hold good.

- (2) *Activity in an unfamiliar or unfavourable environment.* Where this is the case, a single 24-hour catch will seldom yield more than one, or at most two, specimens of the mosquito concerned. It is seldom that a slowly accumulated total, built up in this manner over perhaps as much as a hundred catches, will show a clear pattern of behaviour, while possibly quite a short series, say five or six catches, may show a well-defined biting cycle if the environment is a favourable one.
- (3) *Cases where mosquitos are travelling.* Certain species which show a clear biting cycle in the vicinity of their breeding or resting places, and also at more distant points whither they have gone to feed (*e.g.*, in huts), may in the intervening zone show a biting pattern which, while essentially either nocturnal or diurnal, is otherwise vague and featureless. Probably the impulse to bite is, in such cases, brought about by a close approach to the bait, which has occurred fortuitously during the course of flight. Instances of this type of behaviour are to be found mainly among mosquitos which build up large populations and have a long flight range, such as *Anopheles (Myzomyia) pharoensis* Theo. and those *Mansonia* spp. which have 'omnivorous' habits where blood-meals are concerned. A close parallel may be found in the "induced swarms" discussed by Nielsen & Greve (1950) which have no relation to time or environment, being caused by direct, fortuitous stimulation. Similarly, it is well known that mosquitos may be induced to bite at almost any hour, regardless of their normal periodicity, if disturbed in their resting places by agitation of the foliage, etc. (Haddow, 1945a).

With such points in mind, it is clear that the combination of series from different localities must be undertaken with caution, particularly where more than one level is involved. *Mucidus*, the first subgenus to be discussed, is one of the cases where it seems permissible.

The subgenus *Mucidus*.

The members of this subgenus present a remarkable uniformity in appearance. All are enormous mosquitos, second only to *Toxorhynchites* in size, and their shaggy coat of brown, yellow and white scales is highly characteristic and immediately recognisable. Adults are very rarely taken in catches of resting mosquitos, and are almost equally uncommon in baited catches made at ground-level. As the general appearance of *Mucidus* adults suggests that their coloration is cryptic, it may be that they rest on patches of lichen or moss on trees, presumably at the higher levels.

The Uganda sample here discussed comes exclusively from forest. *Aëdes (M.) nigerrimus* (Theo.) and *A. (M.) grahami* (Theo.) are common and widespread sylvan species, occurring with equal abundance in heavy rain-forest and second-growth. They have occasionally been taken, in the course of routine work, in bush and in plantations also. *A. (M.) scatophagoides* (Theo.) and *A. (M.) mucidus*

(Karsch) are much less common in Uganda, and in 24-hour catches have been represented by only one specimen each. The records given by Edwards (1941) show clearly that in some areas mosquitos of the subgenus *Mucidus* occur in fairly open country, but in Uganda this is not usual.

Together with the four species just mentioned is one which is as yet undescribed and which is here listed as *Aedes* (*Mucidus*) sp.n. A note on this scarce but interesting mosquito is given by van Someren, Teesdale & Furlong (1955) who list Gede and Vanga on the Kenya coast as the only known localities. The writer's specimens, which were identified by Mrs. van Someren, were taken during six catches at Penda Kula, a few miles south of Gede. All were obtained on a platform built just above the mud in a mangrove (*Avicennia*) swamp.

Within the limits of the work here reported, *Mucidus* spp. appear as the most markedly arboreal and nocturnal of East African mosquitos and, as mentioned above, this unusually circumscribed behaviour permits the combination of catch series from different areas. The two species which are well represented, *A. nigerrimus* and *A. grahami*, will now be considered. The data are presented in Table I and fig. 1.

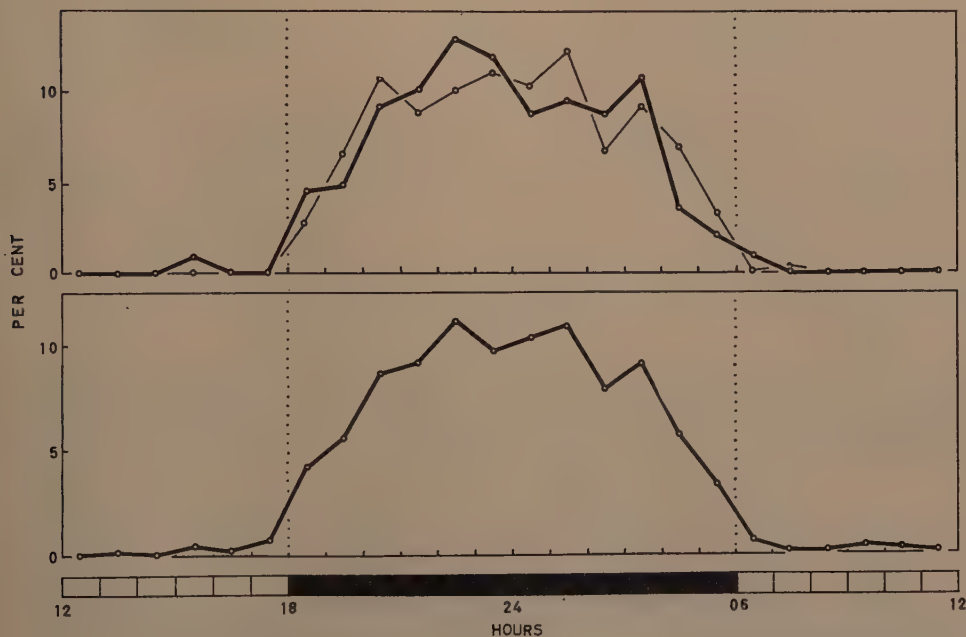


Fig. 1.—Above: the biting cycle of *A. (M.) nigerrimus* (thick line) and *A. (M.) grahami* (thin line) in the forest canopy. Below: the biting cycle of all species of *Mucidus* collectively, regardless of locality or level. M_w has been reduced to a percentage basis to facilitate comparison. The scales have been adjusted so that the greatest ordinate is of the same height in both graphs.

The earliest 24-hour catches to be carried out were a group of 15 made at ground-level in the Semliki Forest, Bwamba (Haddow, 1945a). In this series (First forest catches) no *Mucidus* spp. were taken. Some time afterwards a series of 24-hour catches was made at Mongiro and Mamirimiri in the same forest (Haddow, Gillett & Highton, 1947). At Mongiro there were 20 catches, made simultaneously at four levels and at Mamirimiri there was a similar series involving five levels. The specimens taken in these series were identified as *A. grahami*,

TABLE I.
The biting cycles of species in the subgenera *Mucidus*, *Diceromyia* and *Stegomyia*.

Hour begin- ing	<i>A. scatophagoides</i>		<i>A. mucidus</i>		<i>A. nigerrimus</i>		<i>A. grahami</i>		<i>A. (Mucidus)</i> sp. n.		<i>A. (Mucidus)</i> all spp.		<i>A. furcifer-taylori</i> group		<i>A. adersi</i>		<i>A. fraseri</i>		<i>A. dendrophilus</i>		<i>A. luteocephalus</i>		<i>A. ruwenzori</i>	
	T	M _w *	T	M _w *	T	M _w *	T	M _w *	T	M _w *	T	M _w *	T	M _w *	T	M _w *	T	M _w *	T	M _w *	T	M _w *	T	M _w *
06	—	—	2	6	—	—	—	—	—	—	8	10	—	—	—	—	1	—	4	—	—	—	3	253
07	—	—	—	—	1	—	—	2	—	—	2	3	—	—	—	—	1	—	7	—	—	—	—	—
08	—	—	—	—	—	—	—	—	—	—	2	3	—	—	—	—	1	—	7	—	—	—	—	91
09	—	—	—	—	—	—	—	—	—	—	2	3	—	—	—	—	2	—	7	—	—	—	—	413
10	—	—	—	—	—	—	—	—	—	—	5	3	—	—	—	—	1	—	9	—	—	—	—	148
11	—	—	—	—	—	—	—	—	—	—	3	5	—	—	—	—	—	—	12	—	—	—	—	297
12	—	—	—	—	—	—	—	—	—	—	3	2	—	—	—	—	2	—	7	—	—	—	—	466
13	—	—	—	—	—	—	—	—	—	—	1	2	—	—	—	—	3	—	10	—	—	—	—	216
14	—	—	—	—	—	—	—	—	—	—	2	3	—	—	—	—	—	—	9	—	—	—	—	216
15	—	—	—	—	—	—	—	—	—	—	1	2	—	—	—	—	3	—	9	—	—	—	—	91
16	—	—	2	6	—	—	—	—	—	—	5	8	—	—	—	—	2	—	9	—	—	—	—	365
17	—	—	—	—	—	—	—	—	—	—	3	3	—	—	—	—	2	—	9	—	—	—	—	2296
18	—	—	10	28	7	19	—	—	—	—	47	68	12	—	—	—	—	—	5	—	—	—	—	803
19	1	—	12	30	16	45	—	2	—	—	64	91	3	—	—	—	—	—	—	—	—	—	—	489
20	—	—	20	57	27	73	—	—	—	—	103	142	1	—	—	—	—	—	2	—	—	—	—	216
21	—	—	24	62	22	60	—	4	—	—	109	147	3	—	—	—	—	—	1	—	—	—	—	91
22	—	—	30	80	25	69	—	3	—	—	136	182	1	—	—	—	—	—	1	—	—	—	—	—
23	—	—	30	73	27	75	—	—	—	—	111	159	1	—	—	—	—	—	1	—	—	—	—	—
00	—	—	24	55	26	70	—	1	—	—	140	168	2	—	—	—	—	—	—	—	—	—	—	—
01	—	—	23	59	33	84	—	2	—	—	134	177	1	—	—	—	—	—	1	—	—	—	—	—
02	—	—	22	54	16	46	—	—	—	—	92	130	—	—	—	—	—	—	1	—	—	—	—	—
03	—	—	32	70	22	62	—	—	—	—	109	148	1	—	—	—	—	—	—	—	—	—	—	—
04	—	—	8	22	17	48	—	—	—	—	62	92	1	—	—	—	—	—	1	—	—	—	—	—
05	—	—	5	13	8	22	—	—	—	—	38	54	2	—	—	—	—	—	1	—	—	—	—	148
Totals	1	—	244	—	247	—	—	12	1188	—	—	—	28	31	17	109	7	116	—	—	—	—	—	—

The figures for *A. scatophagoides*, *A. mucidus*, *A. nigerimus*, *A. grahami* and the *A. furcifer-taylori* group refer to catches made in the canopy. Those for *A. (Mucidus)* sp.n. and *A. adersi* refer to catches made just above water-level in a mangrove swamp. In the case of the collective figures for "*Mucidus*, all spp.", all localities and levels have been combined. It will be noted that in this column the total is much larger than the grand total for the species of this subgenus which are shown separately. This is brought about by the inclusion of levels other than the canopy, and of a long series of catches, included in the earlier part of the Bwamba tree survey, in which *A. nigerrimus* and *A. grahami* were not distinguished. In the case of *A. fraseri*, *A. dendrophilus*, *A. luteocephalus* and *A. ruwenzori*, all catches and levels have been combined. T=No. taken; a dash implies that none was taken.

* The figures shown under M_w are, in fact, M_w × 1,000. By this means integer figures can be given.

but later it was found that the identification of *A. grahami* and *A. nigerrimus* had been confused, and that probably both had been represented in the sample. They are here, accordingly, classed as the *A. nigerrimus* group. The results were as follows:—

Level	Mongiro	Mamirimiri
Upper canopy	No catch	—
Main canopy	1	2
Upper understorey	2	3
Lower understorey	3	2
Ground-level	—	—

Thus, though the sample was very small, the arboreal tendencies of the group were clearly shown by the fact that none was taken at ground-level, as opposed to 13 from tree platforms.

Some time after this, a very long series of catches, known as the Bwamba tree survey, was begun. Forty stations were involved, and at each of these six catches were carried out, at ground-level and in the canopy simultaneously. In this survey a large sample of *Mucidus* spp. was obtained, but *A. nigerrimus* and *A. grahami* were not distinguished during the earlier part of the work and so, when comparison is to be made between their biting cycles, only the last 150 catches may be used. The total sample of *Mucidus* spp. obtained in this work may, however, be used to show the general vertical distribution. At ground-level only 64 specimens were taken, while the canopy yielded 1,008 or 94 per cent. of the total. When the figures for the 150 catches in which *A. nigerrimus* and *A. grahami* were distinguished are considered separately, to compare their vertical distribution, the following results are obtained:—

Species	No. taken in canopy	No. taken at ground-level
<i>A. nigerrimus</i>	178 (97%)	5
<i>A. grahami</i>	233 (96%)	9

These mosquitos are thus strikingly similar in their vertical distribution.

Meanwhile Mattingly, working in West Africa (1949*b*), had carried out a series of 22 stratified 24-hour catches, and had obtained an excellent sample of over 1,000 examples of *A. grahami*. His results, where vertical distribution is concerned, agree closely with the Bwamba figures, being as follows:—

Level (ft.)	Percentage of total catch
52	46.8
40	35.4
22	16.4
Ground level	1.4

At Entebbe also, work was in progress and a series of 24-hour catches known as the 'Entebbe tree survey' was carried out, mainly in Zika Forest. Seven stations were used and, at each, 5 catches were made simultaneously in the canopy (at an average height of 57 ft.), in the understorey (at an average height of 35 ft.) and at ground-level. Here the yield of *Mucidus* spp. was very small, but showed the same tendencies as before:—

Level (ft.)	<i>A. nigerrimus</i>	<i>A. grahami</i>
57	2	2
35	—	1
Ground-level	—	—

A series of 60 catches was subsequently made in the canopy at one of these stations, Zika No. IV, and here 64 examples of *A. nigerrimus* and 12 of *A. grahami*

were obtained. In all the localities sampled, therefore, these mosquitoes were markedly arboreal and when the writer's entire collections are considered by level it is found that, for every 10 catch-days, the canopy yielded 22.3 examples of *Mucidus* spp., the understorey 0.1 and ground-level 0.2. The restriction of *A. nigerrimus* and *A. grahami* to the forest canopy is also apparent in some series of 24-hour catches carried out in Bwamba, in various environments simultaneously (Lumsden, 1951). In each case his catching stations were closely grouped and comprised a hut, the clearing round it, a banana plantation, the forest floor and the forest canopy. Lumsden obtained 3 examples of *A. nigerrimus* and 3 of *A. grahami* in the canopy, and none elsewhere.

The sample available for discussion of the biting cycle in *A. nigerrimus* and *A. grahami* consists of the last 150 catches of the Bwamba tree survey, the 35 catches of the Entebbe tree survey and the 60 canopy catches from Zika IV station. Only the canopy figures will be considered. Following the practice of C. B. Williams (1937), a logarithmic transformation—for which the writer (1960) has proposed the name "Williams' mean" (hereafter M_w)—has been used as a measure of the central tendency.

The biting cycle, as is obvious from fig. 1, is strictly nocturnal. Thus, of 244 examples of *A. nigerrimus*, only four were taken by day, and of 247 of *A. grahami*, only one. In both species the highest activity is reached in the middle part of the night, and the correspondence between the two cycles is very close. *A. nigerrimus* appears to show rather more activity before, and *A. grahami* after, midnight. While on the present figures these differences do not appear significant they may well be so, as Mattingly's figures (1949b) show that in West Africa *A. grahami* bites most actively from 23 to 04 hr., but with peak activity toward the end of this period.

The only East African mosquitoes which show really precisely timed peaks of biting activity are those which bite mainly in the crepuscular periods. Apart from such cases, the circadian biting rhythms shown by the two species here discussed are among the most clearly defined of those so far studied. In view of the fact that the behaviour of this subgenus in East Africa seems so circumscribed (see, for example, the figures for *A. (Mucidus)* sp.n. (Table I) in a quite different environment from that just discussed) it has seemed worth while to prepare a single generalised graph for all species combined, regardless of locality or level (Table I and fig. 1). Even where such an all-embracing method is used, the pattern remains very clearly defined, and reference to the table shows that less than 4 per cent. of the total were taken by day. The period of highest activity is the middle four hours of the night, which yielded 44 per cent. of the total catch. In the previous paper in this series the writer pointed out that in some groups of *Aëdimorphus* and in *Neomelaniconion* there seemed to be some indication of a group pattern, where biting behaviour was concerned. This suggestion clearly holds good in the case of the subgenus *Mucidus*.

The number of examples of *Mucidus* spp. taken by other workers in East Africa have been comparatively small. Bailey (1947), working at Gede on the Kenya coast, obtained four examples of *A. scatophagoides* on a tree platform and four at ground-level. In subsequent work, however, where a series of simultaneous catches was carried out on the platform, at the base of the tree and in bush outside the forest, four were taken in the bush, two at the foot of the tree, and none on the platform. These were not 24-hour catches and so probably missed the optimum period. Van Someren, Teesdale & Furlong (1955) also record this species from tree platforms at Gede and from bush, in both of which environments they also took *A. (Mucidus)* sp.n. and *A. mucidus*. Teesdale (1959) carried out stratified 24-hour catches in a mango tree in the Mombasa area and took one specimen of *A. scatophagoides* at 50 ft., and Lumsden (1955a), working further inland at Taveta, took two specimens on a platform at about 16 ft. in bush, during similar

work, but obtained none in the adjoining forest. Subsequently, van Someren, Heisch & Furlong (1958) obtained 30 specimens of this species during 24-hour catches carried out at ground-level. One was taken in a house and the others in bush. These various small samples, when taken together, suggest that *A. scotophagoides* frequents bush and scrub much more freely than do *A. nigerrimus* and *A. grahami* and also that, while it unquestionably does frequent high levels in the trees, it is relatively prevalent at ground-level when compared with these more strictly sylvan mosquitos.

Little is as yet known about the host preferences of *Mucidus* spp. They bite man freely when he is present in the proper environment and at the proper time. Monkeys also are bitten when exposed as baits in the canopy (Haddow & Dick, 1948), and fowls are attacked occasionally (M. C. Williams, personal communication). Even when 24-hour catches are not being carried out, fairly large numbers may be taken quite shortly after sunset, provided a number of platforms with several baits each are used (Haddow & Mahaffy, 1949; Lumsden, 1952; Smithburn, Haddow & Lumsden, 1949). It seems doubtful, however, whether really large populations can ever be built up, the larvae being obligatory predators.

The subgenus *Diceromyia*.

In the work under discussion, this subgenus was poorly represented. The first species to be considered, *A. (D.) adersi* (Edw.) was taken only during the six catches in mangrove on the Kenya coast which have been mentioned above. The results show that it is nocturnal, all 31 specimens having been taken by night, but no particular peak of activity was apparent. Van Someren, Heisch & Furlong (1958) in their 24-hour catches on the Kenya coast took only 11, one in a house and the others in bush. Once again activity was nocturnal. The catches so far discussed were made at ground-level, but it has been shown in other series that *A. adersi* has well-marked arboreal tendencies. Thus van Someren, Teesdale & Furlong (1955) mention that, while females were rare in bush, they were not uncommon on a platform below the canopy at Gede, and Lumsden (1955a) working at Taveta, obtained his single specimen on a tree platform by night. Teesdale (1959) took this mosquito rarely in bush, but in his series of stratified 24-hour catches in a mango tree observed a well-marked arboreal distribution, seven being taken at 50 ft., five at 30 ft. and two at ground-level. The largest sample so far recorded is that obtained by Bailey (1947) who, though he did not carry out 24-hour catches, obtained 327 on tree platforms in a single year at Gede, while at ground-level in bush he took only five females in six years. Clearly, therefore, *A. adersi* is nocturnal and arboreal in its biting habits, though its period of peak activity has not yet been determined.

The remaining members of this subgenus, the group of *A. furcifer* (Edw.) and *A. taylora* Edw., were also poorly represented. These mosquitos, which cannot be distinguished reliably as females, occur over a wide area in East Africa. They occur in many different types of country, ranging from semi-desert to rain-forest. Where they occur they bite man viciously and when abundant may be a plague in the evenings. Their distribution is, however, very patchy, and in many areas it seems to be strongly seasonal also.

In Bwamba, this group was encountered only during the tree survey, only by night and only in the canopy. Though the sample is very small, the biting cycle is clearly defined, 43 per cent. of the total having been taken in the single hour after sunset (Table I & fig. 2). On the Kenya coast, the group also shows arboreal tendencies. Thus van Someren, Teesdale & Furlong (1955) found it rare in bush but common on a tree platform at Gede, and Teesdale (1959), in his stratified catches in a mango tree, took one example at 50 ft. and one at 30 ft., but none at ground-level. Bailey (1947) took 131 specimens on tree platforms at

Gede in one year. His previous six years' catches at ground-level had yielded one only. In catches carried out simultaneously at both levels, he took 39 on a platform and only two at the base of the tree.

In relatively dry and open country, the *A. fuscifer-taylori* group may bite very freely at ground-level. Thus Lewis (1943) found that these species made up more

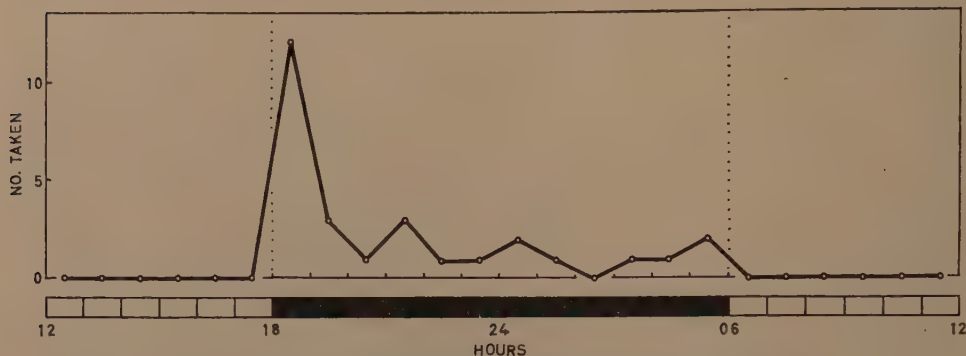


Fig. 2.—The biting cycle of the group of *A. (D.) fuscifer* and *A. (D.) taylori* in the forest canopy (arithmetic totals).

than half of the total in his evening catches in the Nuba Mountains, Sudan (which included no less than 18 species). Similarly the writer, working in arid country at Loyoro, in Karamoja District, Uganda, found them overwhelmingly prevalent in the crepuscular period at ground-level during the rains. In subsequent work at Loyoro, further points of interest emerged. Thus, even in the driest weather, adults could still be found, resting in animal burrows, and they could be taken biting in the evenings in the dense thicket bordering the dry sandy bed of the river. Further, even in such country as this, arboreal tendencies still exist. Thus in five 24-hour catches made at the height of the dry season, six specimens were taken in the early night on a platform at 15 ft. in the river-side thicket, none being taken below. The *A. fuscifer-taylori* group are thus arboreal and crepuscular mosquitoes which will also bite freely at ground-level when forest or bush is scarce or absent, and which are very resistant to drought and heat. It is the common experience of the workers quoted above, and of the present writer, that they rarely enter huts and tents even in areas where they are very prevalent outside.

The subgenus *Finlaya*.

In the Ethiopian Region, the subgenus *Finlaya* is poorly represented. Thus Edwards (1941) lists only eight species for tropical and southern Africa, as opposed to 40 for India, Burma and Ceylon. Only two were taken during the present work, but large samples were obtained in both cases.

The first species, *A. (F.) ingrami* Edw., is essentially a mosquito of second-growth and of the forest fringes. It is not prevalent in heavy forest such as is characteristic of the Bwamba area. In all the series of catches carried out there, only 237 specimens were taken—a mean of well under one per catch. There are cases where a very much smaller sample than this can show a sharply defined result, as in the *A. fuscifer-taylori* biting cycle discussed above. In that instance, however, all the specimens occurred in a comparatively small number of catches and in a limited area. *A. ingrami*, on the other hand, was taken at almost every catching station in Bwamba, but always in ones and twos, and presents an example of that vagueness of biting pattern which often occurs in the cumulation of long

series of results of this type, and which has been discussed above. In the Bwamba catches, the ground-level results showed that biting was diurnal, but nothing more than this can be said. Understorey catches were very few, and the numbers are inadequate for discussion. In the canopy, the cycle was essentially similar to that found in the Entebbe area, but its salient characters were much less clearly marked.

In the catches near Entebbe, large numbers were taken in the light forest which seems to be preferred by *A. ingrami*. The long series in the canopy at Zika No. IV station yielded 875 specimens, but these, having been recorded by single minutes, will be the subject of a separate communication. For the present purpose the three-level catches of the Entebbe tree survey are by far the most suitable. The data are given in Table II and fig. 3.

TABLE II.

The biting cycle of *A. ingrami* in forest, as shown by the Entebbe tree survey, in which 35 catches were made simultaneously in the canopy, the understorey and at ground-level.

Hour beginning	Canopy		Understorey		Ground-level	
	T	M _w *	T	M _w *	T	M _w *
00	2	40	2	40	—	—
01	4	61	2	40	—	—
02	2	40	1	20	—	—
03	3	53	—	—	1	20
04	2	40	3	53	—	—
05	5	104	3	61	2	32
<hr/>						
06	13	247	9	185	1	20
07	5	104	15	287	9	171
08	4	82	1	20	17	291
09	1	20	5	104	9	185
10	1	20	11	129	16	227
11	4	74	11	129	12	191
12	5	68	6	117	5	104
13	1	20	2	40	1	20
14	—	—	6	95	2	40
15	3	61	8	161	6	104
16	7	111	30	474	11	199
17	37	621	100	1774	13	262
<hr/>						
18	22	461	29	514	2	40
19	8	100	1	20	1	20
20	5	104	6	117	1	20
21	2	40	1	20	1	20
22	1	20	2	40	1	20
23	3	53	2	32	—	—
<hr/>						
Totals	140	—	256	—	111	—

T=No. taken; a dash implies that none was taken.

* The figures shown under M_w are, in fact, M_w × 1,000. By this means integer figures can be given.

These results show that in the canopy there is a small peak of activity in the hour after sunrise. An hour later a similar peak is reached in the understorey and an hour later still, at ground-level. These figures could be explained on the assumption that there is a separate population at each level, which begins to bite as the morning light passes a certain threshold value. It is believed, however, that, as previously suggested (Haddow, 1954), *A. ingrami* makes daily vertical

migrations. During the middle part of the day there is comparatively little activity at any level, but in mid-afternoon biting again becomes more active at ground-level and builds up to a peak in the two hours before sunset. At the higher levels the course of events is much more dramatic. Thus, in the understory, the increase in activity begins rather later than at ground-level, and the peak, which is extremely well-defined, is confined to the single hour before sunset. In the canopy, the

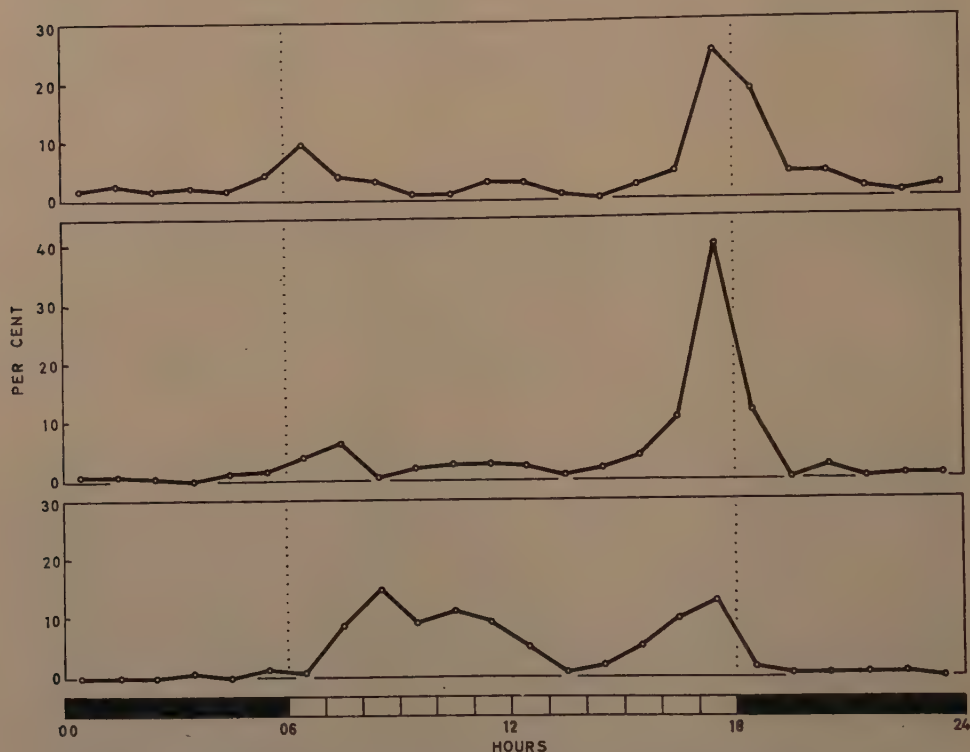


Fig. 3.—The biting cycle of *A. (F.) ingrami* as shown by the Entebbe tree survey. M_w has here been reduced to percentages to facilitate comparison. Top figure, canopy; middle figure, understory; bottom figure, ground-level.

increase begins at about the same time, but here the peak, which is less well-marked than that in the understory, is spread over the hour preceding and the hour following sunset. Thus in the morning the comparatively small peaks occur later and later as ground-level is approached, and in the case of the large evening peaks the process is reversed, the whole being spread over 2–3 hours in each case.

As mentioned above, the evening peak in the canopy shown in the Bwamba tree survey was less well-defined than that observed near Entebbe, and it covered three hours (16–19) instead of two. It is interesting to note, however, that these peak hours yielded 43.5 per cent. of the total canopy catch (as computed from the hourly M_w) and that this figure compares very closely with that given by the two-hour canopy peak in the Entebbe tree survey (42.5%).

Where vertical distribution is concerned, there can be no doubt that *A. ingrami* is an understory species. In the series just discussed the numbers taken were: canopy, 140; understory, 256; ground-level, 111. This distribution has been confirmed by other work in the Entebbe area (Haddow, *in press*). While in this

general area the canopy is apparently preferred to ground-level, this is not always the case. Thus in the 240 catches of the Bwamba tree survey the results were:—canopy, 68; ground-level, 155. Probably here also the understorey would have proved to be the preferred level, but unfortunately it could be sampled only in the catches at Mongiro and Mamirimiri, and in that series the numbers obtained were too small for discussion, only ten specimens having been taken.

Lumsden, in the papers quoted above, obtained under 20 specimens, and van Someren and her co-workers in Kenya even fewer. Mattingly (1949*b*) took only one specimen. As a result, there appear to be no other large series for comparison. There seems to be little doubt, however, that *A. ingrami* is a crepuscular understorey mosquito of the lighter and fringing forest, and, further, that it has the unusual and interesting characteristic of reaching its biting peak at its preferred level in the hour preceding, instead of that succeeding, sunset. The only other mosquito known to the writer which does so is *A. (S.) ruwenzori* Haddow & van Someren (see p. 342).

The second species, *A. (F.) longipalpis* (Grünb.), is even more markedly arboreal than *A. ingrami* and is essentially a diurnal mosquito of the forest canopy. It has the striking metallic markings common in such species (Bates, 1944) and the males do not appear to swarm, whereas those of the more drably-coloured *A. ingrami* are known to swarm over the forest in the sunset period (Haddow & Corbet, *in press*). *A. longipalpis* prefers heavy forest and, though an adequate sample was obtained in the Entebbe area, the only large accumulation comes from the Semliki Forest in Bwamba County. Apart from the series to be discussed below, it may be noted that Lumsden (1951), in his comparative catches in various environments in Bwamba, took 21 in the forest canopy and only one at ground-level. Garnham, Harper & Highton (1946) found that in the Kaimosi Forest the larvae occurred almost exclusively in high tree-holes and they took adults in the canopy, further noting that they were rare at ground-level. *A. longipalpis* is apparently not common in the Lagos area, as Mattingly (1949*b*) took only two specimens, both in the understorey.

In the coastal belt of East Africa this species is replaced by the closely allied *A. (F.) fulgens* (Edw.). Information concerning this mosquito is scanty. Van Someren, Teesdale & Furlong (1955) record it as biting rarely in bush but not in forest; van Someren, Heisch & Furlong (1958) took only one specimen in 24-hour catches in bush; Lumsden (1955*a*) took a small number in bush and forest at ground-level in his work at Gede and Taveta, and Teesdale (1959) records *A. fulgens* as rare in bush. The largest sample appears to be that of Bailey (1947) who, though he obtained larvae up to heights of 20 ft., took all his adult sample of 40 females at ground-level. Thus *A. fulgens*, closely as it resembles *A. longipalpis* in appearance, seems to differ in habits, perhaps preferring bush to forest, and seemingly being relatively common at ground-level.

The first forest catches in Bwamba, carried out at ground-level, revealed the diurnal nature of *A. longipalpis*, as all 25 specimens were taken by day, nine of them in the period 11–14 hr. Similarly, the first stratified catches, those at Mongiro and Mamirimiri, while confirming the diurnal pattern, also showed that *A. longipalpis* is a canopy mosquito. The results are:—

Mongiro		Mamirimiri	
54 ft.	3	82 ft.	8
31 „	2	58 „	17
16 „	2	44 „	5
Ground-level	—	22 „	3
		Ground-level	—

Thus, on the three high platforms, 28 were taken, a mean of over nine per station; on the four understorey platforms, 12 were taken, a mean of three per station, and there was none at ground-level.

In the Bwamba tree survey, a large sample of 916 specimens was obtained. Of these, 904 were taken by day, and only 12 by night (Table III). The arboreal nature of the species was very clearly marked in this series, 859 (94%) being taken in the canopy and only 57 at ground-level. The biting cycle in the canopy shows a steady and rapid increase from shortly after sunrise to a well-marked peak in

TABLE III.

The biting cycle of *A. longipalpis* in forest.

Hour begin- ning	Bwamba tree survey				Entebbe tree survey			
	Canopy		Ground		Canopy		Understorey	
	T	M _w *	T	M _w *	T	M _w *	T	M _w *
00	—	—	—	—	—	—	—	—
01	—	—	—	—	—	—	—	—
02	—	—	—	—	—	—	—	—
03	—	—	—	—	—	—	—	—
04	—	—	—	—	—	—	—	—
05	—	—	—	—	—	—	—	—
06	2	6	—	—	—	—	—	—
07	9	25	1	3	—	—	—	—
08	22	63	4	12	2	32	1	20
09	50	140	3	9	3	53	3	40
10	77	223	7	20	11	161	12	223
11	101	293	7	18	4	82	8	140
12	115	327	5	14	3	61	4	82
13	129	352	8	23	4	74	6	117
14	145	384	9	24	4	82	4	82
15	122	311	10	27	1	20	3	53
16	59	140	1	3	1	20	2	40
17	17	47	1	3	2	40	3	61
18	5	14	—	—	1	20	—	—
19	3	8	1	3	—	—	—	—
20	—	—	—	—	—	—	—	—
21	3	8	—	—	1	20	—	—
22	—	—	—	—	—	—	1	20
23	—	—	—	—	—	—	—	—
Totals	859	—	57	—	37	—	47	—

In the Bwamba tree survey, 240 catches were made simultaneously in the canopy and at ground-level. There were no understorey catches in this series. In the Entebbe tree survey, 35 catches were made simultaneously in the canopy, in the understorey and at ground-level. As only one specimen was taken at ground-level during this survey (in the period 08–09 hr.) this level is omitted from the table. T=No. taken; a dash implies that none was taken.

* The figures shown under M_w are, in fact, M_w × 1,000. By this means integer figures can be given.

mid-afternoon, and a sharp fall thereafter. The biting cycle at ground-level, if allowance be made for the irregularities to be expected in a smaller sample, follows a closely similar trend (fig. 4). The fact that, in the Semliki Forest, *A. longipalpis* was truly at home is shown by the fact that it was taken at every one of the 40 stations included in the Bwamba tree survey. These stations were distributed over more than 20 miles of forest.

In the Entebbe catches, the biting cycle and the vertical distribution were somewhat different. In this area, *A. longipalpis* is far from common, and the writer

was fortunate to obtain 85 specimens in the Entebbe tree survey. Here the vertical distribution was as follows:— 57 ft., 37; 35 ft., 47; ground-level, 1. The arboreal tendency is thus still well-marked, but here there is an apparent preference for the understorey. It is believed that this is not a true preference, but that it is brought about by the lighter and more broken nature of the canopy, which allows the entry of sunshine and wind much more freely than does the dense and gloomy canopy of the Semliki Forest. Thus *A. longipalpis*, which is obviously a marginal species in this area, probably finds itself, when at 50–60 ft. in the Zika area, in a relatively exposed and unfavourable environment, roughly corresponding to the 82-ft. platform at Mamirimiri.

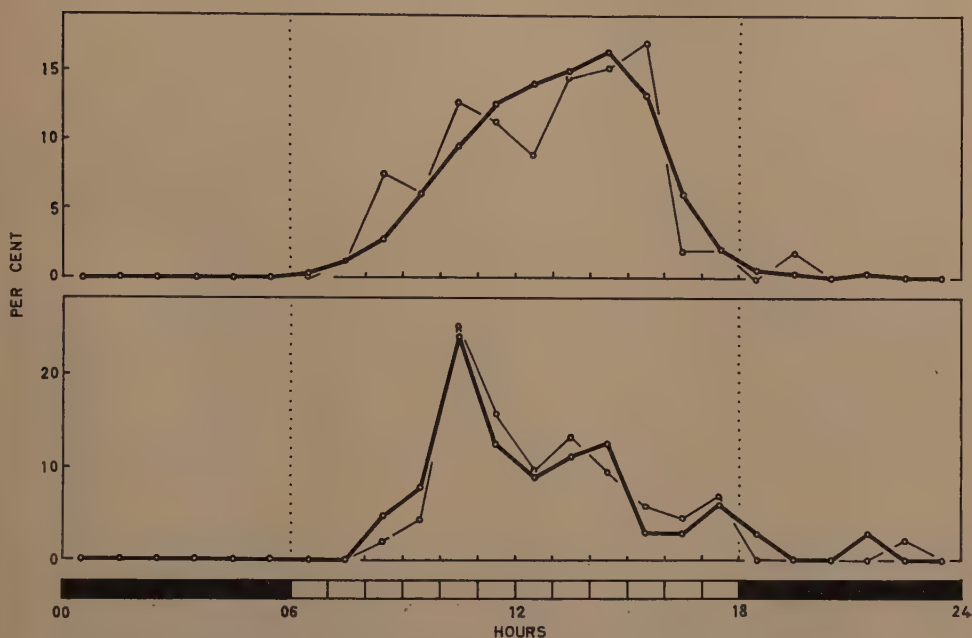


Fig. 4.—The biting cycle of *A. (F.) longipalpis* in forest. Upper figure, Bwamba tree survey (canopy, thick line; ground-level, thin line); lower figure, Entebbe tree survey (canopy, thick line; understorey, thin line). In each figure, M_w has been reduced to percentages to facilitate comparison between levels. The scales are adjusted so that the greatest ordinate in the one is of the same height as the greatest ordinate in the other, to facilitate comparison between the two areas.

The biting cycle in this series shows irregularities which are doubtless due to the small size of the sample. The diurnal nature is maintained, but here both in the canopy and in the understorey the main peak occurs before, not after, midday, both levels following a closely similar trend. The cycle at ground-level in the Entebbe area is unknown, only one specimen having been taken. The reason for the difference in biting cycle from that found in Bwamba (which will here be considered the normal pattern) is not understood. It might be due to a strain difference or to a difference in host. It seems more likely, however, that environmental influence may here play a direct part as Zika Forest, being close to the shore of Lake Victoria, is within the range of the lake breeze which blows on most afternoons and which may well inhibit the biting of this strictly sylvan species at this time. It is admitted that the sample here discussed is a small one, but some confirmation is obtained from the long series of catches at Zika No. IV canopy platform. Only

TABLE IV.
Analysis of the biting cycle of *A. longipalpis* in the forest canopy, as shown by the Bwamba tree survey.

Hour beginning	Biting began at :				Biting ended at :			
	09-10 (on 28 days)		10-11 (on 37 days)		11-12 (on 28 days)		13-14 (on 21 days)	
	T	M _w *	T	M _w *	T	M _w *	T	M _w *
06	-	-	-	-	-	-	-	-
07	-	-	-	-	-	-	1	2
08	-	-	-	-	-	3	5	1
09	33	114	-	-	-	3	1	6
10	19	54	-	-	-	14	9	11
11	22	61	48	122	-	30	10	22
12	24	62	26	51	-	40	11	25
13	33	68	31	53	36	40	14	25
14	39	87	32	59	12	106	23	34
15	41	80	29	55	9	-	41	130
16	22	47	12	22	1	-	-	-
17	3	8	7	12	-	-	-	-
Totals	236	-	211	-	82	-	115	-
					60	-	210	-

The subdivision is into groups of days on which biting began or ended at certain hours. T=No. taken; a dash implies that none was taken. In this table only the daylight hours are quoted as, in the sample discussed, only eight specimens were taken by night.

* The figures shown under M_w are, in fact, M_w × 100, the M_w having been multiplied by 100 to give integer numbers, as in the earlier paper (Haddow, 1954), from which the first three samples are quoted, where the figures were worked out to one decimal place less than in the present series. The second three samples (now presented for the first time) have, accordingly, been worked out to the same number of places.

nine specimens were taken in this series, but all were obtained by day, and six of them before midday.

Perhaps the most interesting feature of both series is the fact that the biting cycle is apparently little affected by level. Thus in the Bwamba tree survey there is an obvious resemblance between the cycle in the canopy and that at ground-level. In the Entebbe tree survey there is a similar resemblance between the two levels which yielded adequate samples, the canopy and the understorey. There is thus no evidence of the vertical movements which seem to occur in *A. ingrami* and this is borne out by the fact that, in routine catches involving enormous samples and covering many types of forest, the writer has always found *A. longipalpis* extremely scarce at ground-level. Whatever may be the controlling impulse in this species, it seems likely that it is basically different from that operating in the case of *A. ingrami*, which, however closely related it may be taxonomically, is in every other way vastly different from *A. longipalpis*, even in such activities as swarming (Haddow & Corbet, *in press*) and egg-laying (Gillett, 1955a).

In a previous communication (Haddow, 1954), an attempt was made to resolve the simple biting cycle shown by the canopy catches of the Bwamba tree survey (fig. 4) into component parts. After a number of methods had been tried, it was found that, if the catches were sorted into groups according to the time at which biting began, an interesting pattern appeared. Three groups of days were used as an example. On days when the first biting activity began in the period 09–10 hr., there was a marked peak of activity in this hour, followed by a sharp decrease and a slow build-up to a minor wave of activity in the early afternoon. When biting began in the period 10–11 hr., the curve was similar, but the initial peak was more pronounced. The afternoon wave remained at the same level and occurred at the same time as before. When biting began in the period 11–12 hr., the initial peak became very marked indeed, while the afternoon wave maintained its former level and position (Table IV & fig. 5).

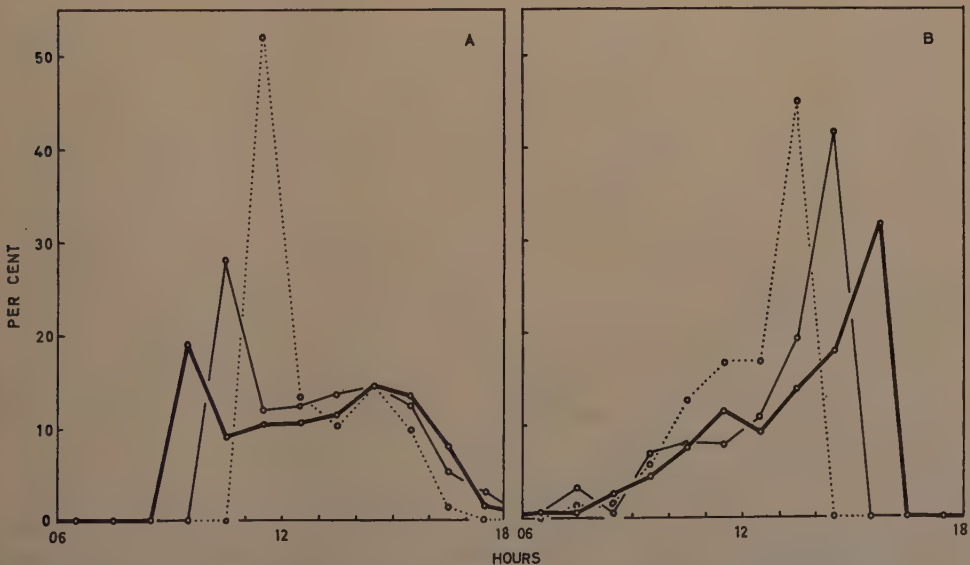


Fig. 5.—The biting cycle of *A. (F.) longipalpis* in the forest canopy (Bwamba tree survey). M_w is used, being reduced to percentages to facilitate comparison. A: thick line, 28 days on which biting began in the period 09–10; thin line, 37 days on which biting began in the period 10–11; broken line, 28 days on which biting began in the period 11–12. B: thick line, 39 days on which biting ended in the period 15–16; thin line, 30 days on which biting ended in the period 14–15; broken line, 21 days on which biting ended in the period 13–14.

It was shown that in these three groups of catches the percentage of the total yield taken before midday remained relatively constant, and it was argued that there were two sections in the population, one biting before midday and the other in the afternoon; the later biting began, the greater the number of the "morning group" which (having been restrained by some environmental factor) would be ready and waiting to bite. This, it was concluded, would account for the manner in which the first wave increases in amplitude the later it begins.

Further study of the figures suggests that these conclusions are not sound. The constant feature of each group is the time at which biting begins. Inevitably this loads the figures in favour of the first hour as, at whatever other times specimens were or were not taken biting, there must have been at least one in the first hour. Thus *A. longipalpis* began biting between 09 and 10 hr. on a total of 28 days. Within this group the largest number of days on which specimens were taken biting at any other given time was 16 (in the period 10-11), a figure only slightly over half that for the first hour. In the case of the 37 days on which biting began in the period 10-11, the highest number of day occurrences at any other given hour was 19 (equally in the periods 12-13 and 13-14) and once again this is only about half the number of occurrences registered for the first hour of activity. Finally, biting began between 11 and 12 hr. on 28 days. The highest number of occurrences for any other time was only nine (in the periods 12-13 and 14-15). In this connection it is most necessary to remember that, where Williams' mean is used, consistency of appearance in the catches is second only to number taken, and it can influence the result greatly when a large number of the records refer to a single specimen.

While the loading of the value for the first hour was inevitable from the method of grouping adopted, the extent of its effect was surprising and was not at first understood. That such loading must occur was accepted from the start, but the fact that the initial peak became higher in each successive group seemed to indicate that some biological reality was also involved, and this led to the conclusions summarised above. It is now necessary to discard these, for the following reasons:— *A. longipalpis* is never a very abundant mosquito, and there are many days on which only a single specimen will be taken. When grouping of days is carried out by the method just described, these single specimens contribute only to the first-hour peak. There are even cases where more than one specimen was taken in the first hour and none subsequently. *A. longipalpis* is a diurnal mosquito, and it is reasonable to suppose that the days on which biting begins late are unfavourable, and that on such days the yield will be small. Further, the unfavourable days will most likely be those on which only a single specimen was taken, or on which biting was confined to a single hour. Inspection of the detailed results confirms this deduction, and shows that the later biting begins, the greater the likelihood that it will be confined to a single hour. Thus in the case of the 28 days on which biting began in the period 09-10 hr., additional specimens were taken at some other time in every case. On the 37 days when biting began in the period 10-11, it was confined to this single hour on four days. Finally, on the 28 days on which biting began in the period 11-12, it was confined to that hour on no less than twelve occasions—approaching half the total.

The conclusion having been reached that the method of analysis was probably unsound, the next stage was to attempt to investigate the matter further. The present argument, being based on the assumption that, on unfavourable days, biting would begin late and would in many cases be confined to a single hour, seemed worth following up from the opposite direction, namely, that on unfavourable days, biting would probably end at an early hour. It was argued that in this case, groupings worked out according to the time at which biting ended should present—roughly—a mirror image of those discussed above. Three groups were taken accordingly, leaving a 1-hour gap between the two series (Table IV & fig. 5).

The results show clearly that a similar trend is followed and that, though the results are far from an exact mirror image, they approach it closely enough to suggest that the original proposition is not sound and that the form of the biting cycles obtained in the particular groups studied was due to the method of selection of the data rather than to any true biological differences in the groups of insects chosen. Thus the former suggestion that there are separate groups in the population of *A. longipalpis*, biting at different times, can no longer be considered as receiving support from the present sample.

The subgenus *Stegomyia*.

According to Edwards (1941) this subgenus is characteristically African. Already 34 species, 2 subspecies and several varieties have been recorded from the Ethiopian Region, and it seems unlikely that the list is anything like complete. Of unquestioned importance in the transmission of virus infections, the subgenus includes many of the most handsome and interesting of the African Culicines, and is notable for the number of species which have arboreal tendencies. Among those studied by the writer, only one—the inappropriately named *A. (S.) dendrophilus* Edw.—has shown a marked preference for ground-level.

The writer's series of *A. (S.) aegypti* (L.) has been derived almost exclusively from routine or short-duration catches. The biting cycle has, however, been discussed by Teesdale (1955), Lumsden (1957), van Someren, Heisch & Furlong (1958) and McClelland (1959, 1960). While results obtained in huts have been variable, most of the outdoor work has shown a pronounced wave of biting in the late afternoon. That this species has arboreal propensities where oviposition is concerned has been shown by Harris (1942), that it will bite in the forest canopy, by Haddow & Mahaffy (1949) and in isolated trees, by Teesdale (1955). It does seem, however, to bite and oviposit much more freely at the lower levels.

One of the commoner East Africa species, *A. (S.) apicoargenteus* (Theo.), will not be discussed in detail here, as the results for all the writer's catches have been described elsewhere (Haddow, *in press*). This is essentially a mosquito of broken forest and second-growth (Garnham, Harper & Highton, 1946) though it may also be common in rain-forest. Its preferred zone, where biting is concerned, is the understorey and lower canopy, though, where oviposition is concerned, high tree holes are the most heavily colonised (Corbet, *in press*). The biting cycle is strictly diurnal and rather irregular. In most series of catches (*e.g.*, those described by Haddow, Gillett & Highton, 1947) the highest activity has been attained in the afternoon.

The other species may now be discussed:—

A. (S.) simpsoni (Theo.) breeds mainly in banana, colocasia and pineapple axils (Teesdale, 1941; Gibbins, 1942; Haddow, 1948), though tree holes are also used (Dunn, 1926; Wiseman & others, 1939; Haddow, van Someren & others, 1951). Under urban conditions it shows a dangerous tendency to adopt peri-domestic breeding habits, utilising artificial containers (Wiseman & others, *l.c.*; Muspratt, 1956). In some areas it bites man freely (Gibbins, 1942, Mahaffy & others, 1942; Haddow, 1945a; Lumsden, 1955a; Teesdale, 1959) while in others it does not seem to attack man at all (Kerr, 1933; Gillett, 1951a, 1955b; Boorman & Porterfield, 1957). A short review of available information on this subject (up to the early fifties) is given by Mattingly (1952).

All the workers quoted have found *A. simpsoni* to be a mosquito which rarely enters houses, and all have found it to be markedly diurnal in its biting habits, the only record of nocturnal activity being that of Bedford (1928). The biting cycle, as seen in the writer's first series of 24-hour catches in banana plantations (Haddow, 1945a) showed a good deal of activity in the morning, a poorly defined lull just before noon, and a major wave of activity in mid- to late afternoon. These

figures (Table V & fig. 6) have not been published previously on an hour-to-hour basis. Subsequently, a series of catches was made simultaneously at ground-level, 6, 12 and 18 ft. in a banana plantation. The results (Haddow, 1945b) were published on an hourly basis for all levels collectively and on a 4-hourly basis for each level separately. Subsequent analysis of the figures shows that it is preferable to divide them into two groups, those for ground-level and 6 ft., which are similar, and those for 12 and 18 ft., which are similar. The results (Table V &

TABLE V.
The biting cycle of *A. simpsoni* in Bwamba.

Hour begin- ning	First plantation catches		Stratified plantation catches				Forest	
	(Ground)		(Ground + 6')		(12' + 18')		(All levels)	
	T	M _w *	T	M _w *	T	M _w *	T	M _w *
00	3	127	—	—	—	—	—	—
01	—	—	—	—	—	—	1	2
02	—	—	—	—	—	—	—	—
03	—	—	—	—	—	—	—	—
04	3	96	—	—	—	—	—	—
05	5	259	—	—	—	—	1	2
06	64	2587	3	148	6	349	—	—
07	90	4346	19	1163	12	698	4	12
08	92	4684	30	2076	21	1360	3	7
09	95	4555	30	2048	25	1979	4	12
10	100	4666	29	2112	28	2048	6	16
11	59	3378	36	2606	29	2133	5	14
12	89	4666	30	1805	31	2034	9	26
13	78	4330	21	1296	21	1244	18	42
14	102	6134	31	1979	22	1606	13	35
15	92	4728	33	2373	27	1679	21	52
16	104	5781	39	2855	24	1339	11	28
17	74	3966	34	2396	12	762	8	19
18	7	294	3	148	—	—	5	14
19	2	96	—	—	—	—	1	2
20	3	127	—	—	—	—	2	5
21	—	—	—	—	—	—	—	—
22	—	—	—	—	—	—	—	—
23	2	76	—	—	—	—	—	—
Totals	1064	—	338	—	258	—	112	—

The first plantation catches comprised 15 catches made at ground-level. In the stratified plantation catches, 10 were made at ground-level, 6 ft., 12 ft. and 18 ft. simultaneously. Here the two lower and the two higher levels have been combined. The forest series is made up by 15 ground-level catches (the first forest catches) and the 240 catches of the Bwamba tree survey, made simultaneously at ground-level and in the canopy. No specimens were taken in the Mongiro-Mamirimiri series. T=No. taken; a dash implies that none was taken.

* The figures shown under M_w are, in fact, M_w × 1,000. By this means, integer figures can be given.

fig. 6) again show a biphasic cycle, though this time the trough occurs some time after midday. At 12 and 18 ft., the greater wave is in the morning. At ground-level and 6 ft., the wave in the morning is not much below the amplitude (when considered on a percentage basis) of that at the higher levels, but that in the afternoon is much greater. Thus both in the first and the second series the

maximum incidence of biting at ground-level was in the afternoon. The failure of the afternoon peak to develop at the higher levels is thought to be due to the inhibitory effect of afternoon breezes blowing from the north spur of Ruwenzori, close to which the plantation concerned was situated, and which selectively affect the higher and more exposed levels. It may be mentioned that in the earlier discussion of the cycles at different levels, where 4-hour grouping was employed

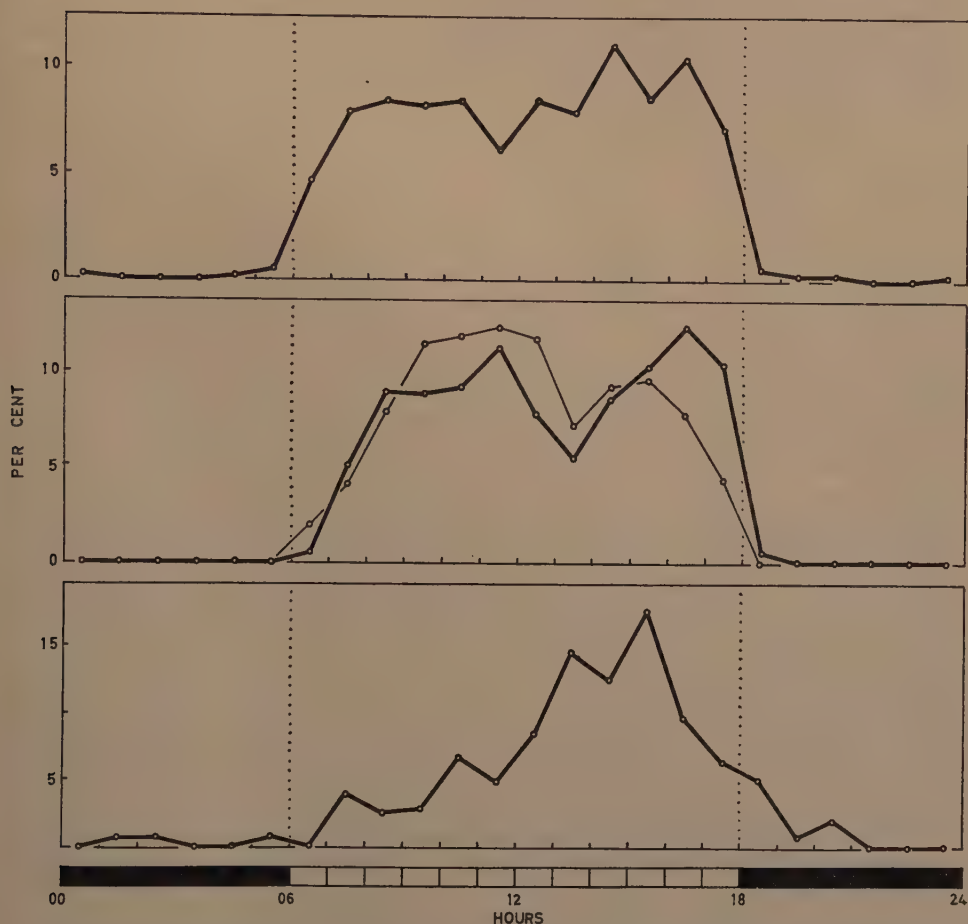


Fig. 6.—The biting cycle of *A. (S.) simpsoni* in Bwamba. Top figure, first plantation catches (15 catches at ground-level); middle figure, stratified plantation catches (10 catches made simultaneously at four levels); thin line, catches at 18 ft. and 12 ft. combined; thick line, catches at 6 ft. and ground-level combined; bottom figure, all forest catches, regardless of level (295 catches). M_w has here been reduced to percentages to facilitate comparison between levels in the middle figure. The scales have been adjusted so that the greatest ordinates in each graph are of the same height, to facilitate comparison between series.

(as coinciding best with the successive climatic phases of the diel) the biphasic nature of the curves was not apparent. In 24-hour catches on the Kenya coast, Teesdale (1959) found a similar biphasic cycle, but here the peaks were later still—one just after noon and the other just before sunset. His numbers, however, are small, and in other catches in bush, confined to the hours of daylight, he found

the lull occurring before midday. Thus having regard to the variability of behaviour in this species, it is felt that all that can be said is that at ground-level or slightly above it, in environments outside forest, the cycle is diurnal and reaches its highest level in the afternoon. It may be biphasic, but this is far from certain as in each series of catches the position of the lull is different, varying from late morning to mid-afternoon. The position of the afternoon peak is more constant, usually occurring 2-3 hours before sunset. Teesdale's bush catches are given in the form of an index (mosquitos per man-hour) as not the same amount of catching was done at all times of day. Knowing the total size of the sample, however, it is possible to work out from the indices the expected number of mosquitos per hour in this series. If all Teesdale's series and those of the present writer are now combined, a curve for diurnal biting can be constructed, the results being:—

Hour beginning	Percentage of total catch	Hour beginning	Percentage of total catch
06	8.0	12	8.0
07	5.4	13	8.6
08	5.6	14	9.7
09	6.1	15	9.2
10	8.8	16	12.3
11	8.2	17	10.0

This curve is of course a very crude one, as it makes no allowance for weighting of the result by the larger samples. It is an interesting set of figures, none the less, as it closely resembles in trend the biting cycle of *Anopheles (Myzomyia) gambiae* Giles, though this is of course a nocturnal species, while *A. simpsoni* is diurnal.

In forest, the cycle (Table V & fig. 6) is very different, showing a smooth rise from a low morning level of activity to a clearly defined peak in the third hour before sunset. Lumsden (1955a) found a very similar cycle in forest at Taveta, Kenya, both at ground-level and on a tree platform. It is most interesting that he found the same type of cycle in banana plantations at Taveta, as these small plantations are in the heart of the forest, and are shaded by the huge trees on all sides. This well-defined cycle, with a single marked peak in the late afternoon, may thus perhaps be taken as characteristic of the forest habitat. In Bwamba, as at Taveta, the cycle in the canopy and at ground-level in forest was similar.

A. simpsoni does not penetrate far into forest in East Africa (Haddow, 1945a) but when it does occur it is almost as prevalent in the canopy as it is at ground-level. Thus Lumsden's catches at Taveta yielded 133 from the platform and 188 from ground-level. Working in Bwamba (1951) he obtained nine in the canopy and ten at ground-level in simultaneous catches, and the writer (1950) took 42 in the canopy as opposed to 47 at ground-level, also in Bwamba.

Two of Lumsden's Bwamba series were made simultaneously in five different but very closely contiguous environments, and show clearly the preference of *A. simpsoni* for banana plantations. His results were as follows:—

Hut	0
Open space round hut	13
Banana plantation	245
Forest floor	10
Forest canopy	9

Even within the banana plantation there is localisation, the writer's (1945a) results being:—

Road through plantation	3
Village clearing in plantation	7
Dense weedy area with tall plants	11
Sparse, recently planted area	75
Edge of plantation	165

In the same paper it is noted that *A. simpsoni* apparently prefers human blood, and that it attacks the head selectively, even when the bait is sitting or lying, a fact previously noted by Gibbins (1942). It seems to bite most freely in warm overcast weather (Haddow, 1945*b*) and is one of those species which attacks in waves at short intervals, a phenomenon which has been discussed by Haddow (1954) and very clearly demonstrated by Colless (1956) in other mosquitos.

A. (S.) fraseri (Edw.) is far from common in Uganda, except in the Semliki Forest, where fair numbers have been taken during routine catches, though very few in 24-hour catches. None was obtained in the first series of catches in forest, and only one in the Mongiro-Mamirimiri series. In the Bwamba tree survey, 17 were taken, 6 in the canopy and 11 at ground-level. While this small Bwamba sample seems to indicate a preference for ground-level, it is to be remembered that Garnham, Harper & Highton (1946) found it more abundant at 55 ft. than at ground-level in the Kaimosi Forest, where it is prevalent, and also recorded breeding up to a height of 60 ft. Little can be said about the biting cycle, except that it is obviously diurnal (Table I).

A. (S.) dendrophilus is also rather uncommon in Uganda, except in the Semliki Forest. In the past there was considerable confusion concerning the taxonomy and nomenclature of the group to which this mosquito belongs, owing to a transposition in a figure legend in Hopkins' standard work on the larvae of Ethiopian Culicines (1936). As a result, it was referred to *A. (S.) deboeri* subsp. *demeilloni* Edw. in the writer's earlier papers. The matter has been reviewed by van Someren (1946) and by Smithburn, Haddow & Gillett (1948). No specimens were obtained in the earliest catches in Bwamba, and only four in the Mongiro-Mamirimiri series. In the Bwamba tree survey, 105 were obtained, 80 at ground-level and 25 in the trees. There is thus a distinct preference for ground-level. The biting cycle is markedly diurnal, but otherwise there is little sign of a definite pattern, and for present purposes all series and levels have been combined (Table I). Most of the sample was made up by single specimens, four being the largest yield on any single day, and the featureless nature of the cycle is a good example of what often occurs when a sample is built up slowly in this manner (see p. 318).

A. (S.) africanus (Theo.) is among the most interesting and important of the Ethiopian Culicines where the transmission of virus infections is concerned. It has long been known that its biting activity is crepuscular (Kerr, 1933; Haddow, 1945*a*; Haddow, Gillett & Highton, 1947; Haddow & Mahaffy, 1949; Mattingly, 1949*a*; Lumsden, 1952). The fact that it is predominantly a canopy species was first shown by Haddow, Gillett & Highton (*l.c.*), since when much of the work on this mosquito has been concentrated on the upper forest levels. Throughout the writer's work the behaviour of *A. africanus* has been highly consistent, and it is considered justifiable to combine all series, level by level, though, in the table, the results for each main series are also shown separately for reference purposes (Table VI & fig. 7).

In the canopy, the preferred zone, there is a single very clearly marked peak in the hour after sunset. In the understorey the over-all pattern is the same, but the peak is very much less well-marked. At ground-level, however, the pattern is very vague and most of the biting occurs by day, when 70 per cent. of the total yield was obtained. This different ground-level pattern was apparent in the early catches at Mongiro and Mamirimiri (Haddow, Gillett & Highton, *l.c.*), but the sample was considered to be too small to permit conclusions to be drawn. Mattingly (1949*a, b*) noted the same type of cycle at ground-level, but here the picture was confused by the presence of the closely allied *A. (S.) pseudoafricanus* Chwatt (Chwatt, 1949) which is more of a ground-haunting species than is *A. africanus*. The subsequent series have, however, confirmed that, at ground-level, *A. africanus* shows a very vague cycle, with a preference for the hours of daylight.

There have been, nonetheless, some cases of divergence from the pattern

TABLE VI.

The biting cycle of *A. africanus* in forest.

Level		Canopy						Understorey				Ground level					
Series		M.M.	B.T.S.	E.T.S.	Z.IV.	All	M _w *	M.M.	E.T.S.	All	M _w *	F.F.C.	M.M.	B.T.S.	E.T.S.	All	M _w *
No. of catches		60	240	35	60	395	—	80	35	115	—	15	40	240	35	330	—
06-07	..	4	46	25	13	88	129	5	15	20	104	—	4	—	6	10	18
07-08	..	4	41	17	5	67	109	4	8	12	58	—	2	2	5	9	17
08-09	..	2	28	14	—	44	58	—	18	18	71	—	4	7	12	23	32
09-10	..	1	19	11	—	31	51	2	16	18	77	—	1	9	25	35	43
10-11	..	—	17	13	1	31	46	5	9	14	99	—	2	4	26	32	39
11-12	..	—	11	8	1	20	29	2	11	13	67	—	—	8	13	21	36
12-13	..	1	13	6	5	25	39	2	7	9	47	—	—	12	18	31	48
13-14	..	1	13	8	1	23	36	2	7	9	55	—	3	17	10	30	54
14-15	..	1	14	12	2	29	44	2	10	12	59	—	2	12	10	24	45
15-16	..	2	13	11	1	27	43	2	7	9	45	2	—	12	7	21	39
16-17	..	1	28	11	2	42	66	1	18	19	82	—	1	20	5	26	44
17-18	..	10	161	50	42	263	364	6	66	72	199	3	2	21	11	37	74
18-19	..	149	688	326	592	1755	2125	46	133	179	720	3	—	16	16	35	56
19-20	..	57	279	126	206	668	840	19	37	56	285	—	—	9	10	19	37
20-21	..	17	165	97	116	395	525	13	37	50	260	—	—	11	2	13	28
21-22	..	10	133	71	148	362	467	6	35	41	152	3	—	4	3	10	19
22-23	..	11	102	78	78	269	358	5	21	26	140	—	1	7	3	11	22
23-24	..	7	87	62	45	201	268	5	25	30	154	1	—	4	2	7	14
00-01	..	2	66	44	19	131	192	3	19	22	115	—	—	6	—	6	12
01-02	..	4	57	42	15	118	168	1	10	11	69	1	—	5	2	8	17
02-03	..	4	46	27	12	89	133	4	16	20	101	1	—	3	1	5	10
03-04	..	1	41	24	12	78	121	4	12	16	89	—	—	1	1	2	4
04-05	..	—	43	15	15	73	101	2	9	11	59	—	—	3	1	5	9
05-06	..	6	67	17	17	107	156	5	16	21	103	—	—	2	4	6	13
Totals	..	295	2178	1115	1348	4936	—	146	562	708	—	15	22	195	194	426	—

The arithmetic totals for each series are given separately but M_w has been worked out only for the combined samples. F.F.C., first forest catches; M.M., Mongiro-Mamirimiri catches; B.T.S., Bwamba tree survey; E.T.S., Entebbe tree survey; Z.IV, Zika No. IV station canopy series. A dash implies that none was taken.

* The figures under M_w are, in fact, M_w × 1,000. By this means, integer figures can be given.

described above. Thus in a series of catches made at ground-level in a banana plantation in Bwamba, *A. africanus* showed the post-sunset peak usually characteristic of the upper forest levels (Haddow, 1945a). It was concluded subsequently that the reason for this was that the plantation concerned was situated on the lip of a semi-precipitous ravine, choked with heavy forest. The catchers were thus within a few yards of the crowns of large forest trees, though themselves seated at ground-level, and it was considered that the mosquitoes were perhaps merely diffusing outward from the forest canopy into the fairly dense vegetation of the

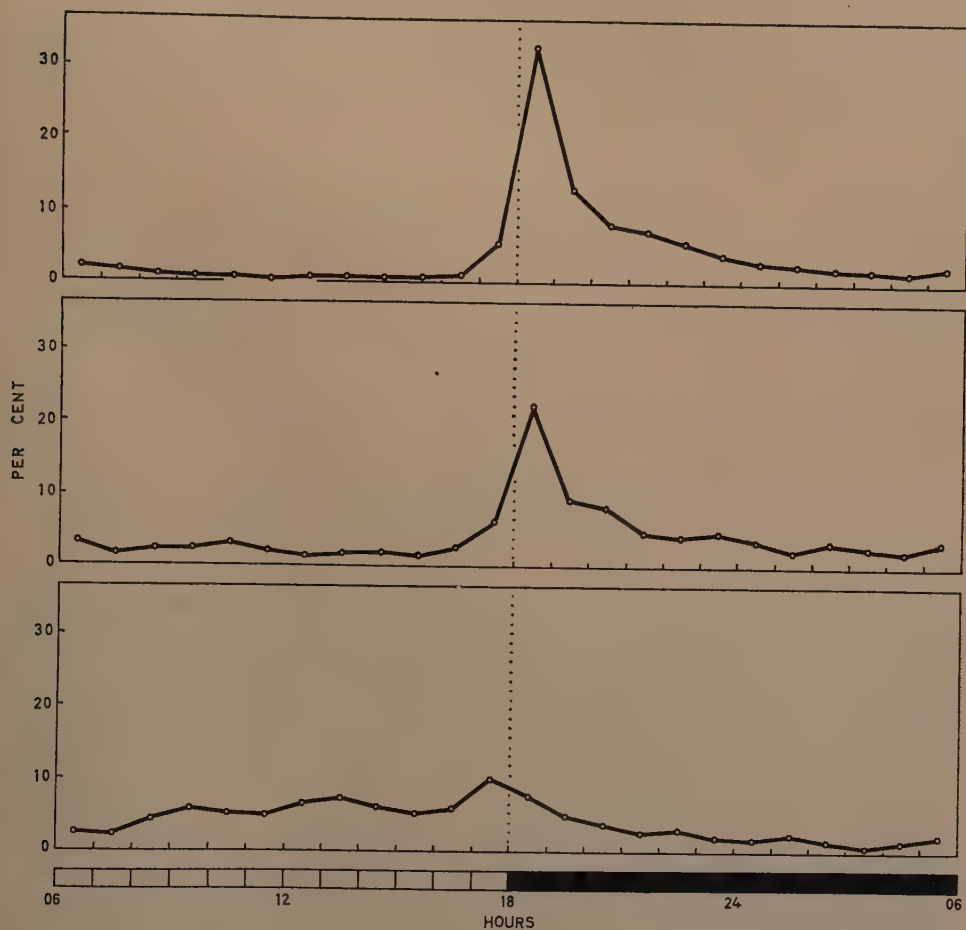


Fig. 7.—The biting cycle of *A. (S.) africanus* by hour and level. All series combined. Top figure, canopy; middle figure, understorey; bottom figure, ground-level. M_w has here been reduced to percentages to facilitate comparison between the levels.

plantation. It may be, however, that *A. africanus*, when outside forest, normally does show a crepuscular peak at ground-level. Thus apart from the plantation catches just described, Kerr's original series showed pronounced crepuscular activity, and he notes that "In Nigerian villages natives of all ages are abroad in the cool of the early evening, just at the time when these mosquitoes are most active."

One set of results diverges widely from experience elsewhere. Working on a platform in Taveta Forest in Kenya, with controls at ground-level, Lumsden (1955a) took 11 specimens at 42 ft. and 8 at ground-level. All the ground-level specimens were taken by day, which was not unexpected. The surprising thing was that 10 of the 11 obtained on the platform were also taken by day. The total sample is of course very small, and whereas Lumsden states that "Although the . . . platform was only about half the height of the average trees it was well situated among foliage on the lower edge of the main canopy", the writer, who has seen the platform, would describe it as in the understorey and in a rather exposed spot—not a station from which he personally would have expected to obtain many specimens. Lumsden's results, however, are so different from anything described elsewhere that it is felt that further work at Taveta is needed to settle the point.

Where vertical distribution is concerned, *A. africanus* is always, within the writer's experience, markedly arboreal. In fig. 8 are shown the results for the

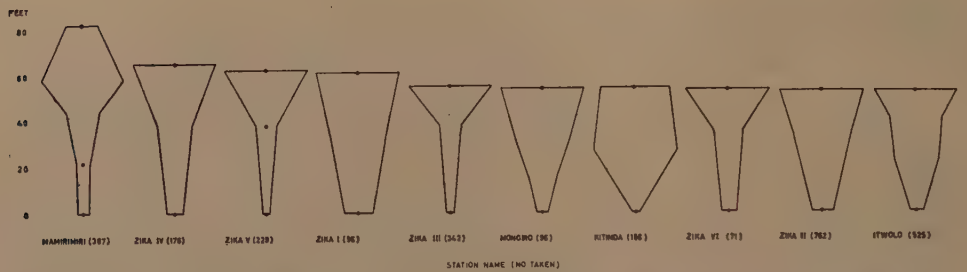


Fig. 8.—The vertical distribution of *A. (S.) africanus* at 10 stations (figures for all stations except Itwolo reduced to M_w). The scales have been adjusted so that the maximum width is the same in each graph.

nine stations at which catches have been carried out at three or more levels simultaneously, together with Mattingly's results from Itwolo near Lagos. In the case of the first diagram (Mamirimiri) the main canopy level is at 58 ft., and the 82-ft. result was obtained in the crown of a semi-emergent tree. There is thus apparently a decrease in numbers above the main canopy. This may not always be the case, however, as is shown by the results of catches on a 120-ft. steel tower in Mpanga Forest, Uganda (Haddow, *in press*). Unfortunately *A. africanus* was extremely scarce at Mpanga, only 16 being taken. Of these, however, eight were obtained above the canopy (7 at 90 ft. and 1 at 120 ft.). Further work on this subject is projected, as it is hoped to move the tower to Zika Forest, a more favourable locality. In all the localities shown, except Kitinda, the canopy shows a higher catch than the understorey. The Kitinda station was, however, within a few yards of the shore of Lake Victoria, and was much more exposed to breezes in the evening. Examination of the five catches made there shows that the apparent impartiality of *A. africanus* as between the canopy and understorey at that station was purely due to inhibition of biting at the higher level by evening breezes. On the single calm evening the distribution was of the usual type. The results were as follows:—

Catch	1	2	3	4	5
Sunset breeze	—	+	++	++	++
No. { Canopy	51	25	9	9	3
taken { Understorey	18	23	17	17	7
{ Ground-level	1	2	2	—	2

To investigate this effect further, the entire 35 catches of the Entebbe tree survey were now examined in this manner (fig. 9).

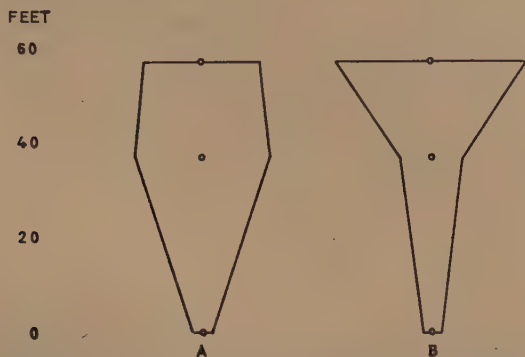


Fig. 9.—The vertical distribution of *A. (S.) africanus* in the Entebbe tree survey on evenings (A) with and (B) without breeze at sunset (M_w as percentage).

In the diagram, the canopy and understorey levels are shown at the mean value for the seven stations (57 and 35 ft., respectively). The results were:—

Wind at sunset — 9 days. Mean catch 59.			
Level	No. taken	100 M_w	M_w as %
Canopy	214	1539	43.1
Understorey	225	1776	49.8
Ground-level	88	254	7.1
No wind at sunset — 26 days. Mean catch 52.			
Level	No. taken	100 M_w	M_w as %
Canopy	901	2477	70.7
Understorey	337	791	22.6
Ground-level	106	234	6.7

Thus while the mean size of the catch was very similar in the two groups of days, the vertical distribution was very different, many examples of *A. africanus* being driven down from the canopy to the understorey and perhaps even to ground-level on windy evenings. This is most clearly shown when the catching rates for the two groups are compared, as follows:—

Level	Yield per 100 man-hours	
	Sunset breeze	No breeze
Canopy	33.0	48.1
Understorey	34.7	18.0
Ground-level	13.6	5.7

Finally it may be noted that, like some of the other species here discussed, *A. africanus* apparently makes daily vertical migrations. In fig. 10 the distribution is shown throughout the diel, by 4-hour groups. During the day the distribution is almost equal at all levels, with a hint of downward movement in the middle of the day and a slight rise in the afternoon. After dark, however, the mosquitoes rise to the canopy and there is only moderate activity in the understorey, and very little at ground-level.

A. (S.) luteocephalus (Newst.) is very poorly represented in the writer's 24-hour collections, being a species which is most prevalent in country drier than that in which the present work has been carried out. Only seven specimens have been taken in 24-hour catches, all in the forest canopy and all by night. In view of the

close relationship between this species and *A. africanus*, this strong arboreal tendency is not surprising. A crepuscular periodicity is suggested by material collected during routine evening catches.

The biting cycle has been more adequately studied by Kerr (1933) who found a pattern extremely similar to that of *A. africanus* with a sharp crepuscular peak in the hour after sunset. Subsequently Lumsden & Buxton (1951) obtained a similar result in West Nile District, Uganda, but the peak as observed by them was longer, being spread over the 2-hour period following sunset. Like the present writer, they found *A. luteocephalus* to be arboreal, only two of their 24 specimens having been taken at ground-level. It is to be remembered, however, that all Kerr's catches were carried out at ground-level.

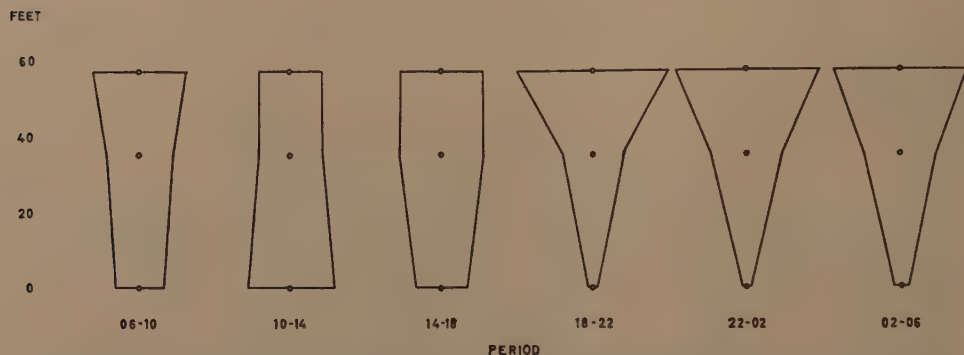


Fig. 10.—The vertical distribution of *A. (S.) africanus* in the Entebbe tree survey at different times of day and night (M_w as percentage).

A. (S.) ruwenzori was discovered by the writer in the cloud-forest of Ruwenzori (Mountains of the Moon) in 1947, and was described by Haddow & van Someren (1950), while the male and immature stages were described by Gillett (1951b). In the first catches it was noted that, like *A. africanus* and *A. luteocephalus* to which it is probably related, this species has crepuscular and arboreal tendencies. The catches on which these conclusions were based were, however, of short duration, and they were not divided into 1-hour groups. It was not till 1960 that an opportunity arose to make 24-hour catches in the Ruwenzori Forest. The zone in which *A. ruwenzori* is most prevalent, from 5,000 to 7,000 ft., is extremely wet, cold and stormy, and during the present series of eight catches the weather was particularly bad, with several hailstorms in the afternoons and evenings. The hardy nature of *A. ruwenzori* was shown by the fact that even after these storms it was on the wing and could be taken attempting to bite, though obviously too chilled to do so effectively. In bad weather of this nature it was forced down to ground-level much in the manner described above for *A. africanus*, but the earlier work has shown that on fair evenings it bites almost exclusively in the trees. The catches were carried out on platforms, with controls at ground-level, and, in the present series, 68 were taken in the trees and 48 at ground-level. The earlier work, carried out in more favourable weather, gave a tree:ground catch ratio of about 3:1.

The biting cycle at both levels was very similar, and for present purposes they have been combined (Table I and fig. 11). It shows a single well-defined peak of activity which occurs, like that of *A. ingrami*, in the hour before sunset, after which a decrease to zero occurs quite rapidly (the later night hours in the Ruwenzori Forest are always very cold, and probably the temperature falls below the flight threshold for *A. ruwenzori*). There is a little desultory biting by day.

Preliminary work suggests that *A. (S.) bambusae* Edw. and *A. (S.) angustus* Edw., which occur at even higher levels (8,000–9,000 ft.) in the cold bamboo forests of Chuya Ridge and the Birunga Volcanoes on the Congo border, are also arboreal and crepuscular (Haddow & van Someren, 1950) but it has not yet been possible to carry out 24-hour catches in that area.

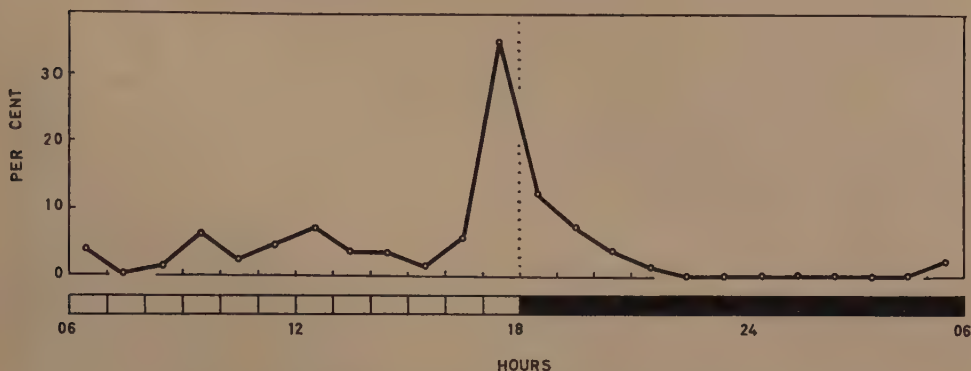


Fig. 11.—The biting cycle of *A. (S.) ruwenzori* regardless of level (M_w as percentage).

The medical importance of the four subgenera.

As in the case of the three subgenera discussed in the previous paper (Haddow, 1960), several of the viruses here discussed were isolated incidentally, during the course of studies on the epidemiology of yellow fever in East Africa.

No virus has been isolated so far from *Mucidus* spp. The same applies to the subgenus *Diceromyia*, though here Lewis (1943) considered that members of the *A. furcifer-taylori* group were probably very important vectors during the severe yellow-fever epidemic which occurred in the Nuba Mountains in the Sudan in 1940 (Kirk, 1941). In addition to Lewis's view, which depends upon epidemiological evidence collected locally, it has been shown that *A. taylori* is capable of transmitting the virus by bite under laboratory conditions (Lewis, Hughes & Mahaffy, 1942).

In the case of the subgenus *Finlaya* the position is a little clearer, though still uncertain. It is considered likely that in the African rain-forest there may be a diurnal, arboreal vector of yellow fever, and that this may be *A. longipalpis* (Haddow, Dick & others, 1951) but no laboratory transmission experiments have as yet been carried out with this species. On the other hand, it is extremely likely that Uganda S virus (Dick & Haddow, 1952) was isolated either from this species or from *A. ingrami*, as the infected lot of mosquitos consisted of *A. longipalpis*, 47 examples, *A. ingrami*, 17 and *A. natronius*, 1. One example of *A. ingrami* was also included in the very large mixed lot of *Aedes* spp. from which Bunyamwera virus was isolated (Smithburn, Haddow & Mahaffy, 1946) but there is good reason to suppose that the infected species in this lot was probably *A. (Neomelanicion) circumluteolus* (Theo.) (Haddow, 1960).

The subgenus *Stegomyia* is of course highly important in relation to virus transmission in the equatorial belt of Africa. *A. aegypti*, the classical vector of yellow fever, seems to be of more importance in West than in East Africa in this respect. The first isolation of yellow-fever virus from this species in Africa was made by Beeuwkes & Hayne (1931) at Ife, in Nigeria, and isolations have been made also by Mattingly at Ogbomosho, in Nigeria (Macnamara, 1955). In East Africa the most important rôle played by *A. aegypti* so far has been that of vector in the very severe epidemic of chikungunya, a dengue-like disease, which occurred

L Edw.

in southern Tanganyika in 1952-53 (Robinson, 1955; Lumsden, 1955*b*; Ross, 1956). In addition to the above, *A. aegypti* is well known as a vector of dengue (Bancroft, 1906; Cleland, Bradley & McDonald, 1916), and in the laboratory it has been shown to be capable of transmitting many other viruses, including West Nile virus (Davies & Yoshpe-Purer, 1954*a*), Zika virus (Boorman & Porterfield, 1956), Uganda S virus (Boorman, 1958) and Semliki Forest virus (Davies & Yoshpe-Purer, 1954*b*; Woodall & Bertram, 1959), all of which were originally isolated by members of the staff of this Institute.

A. apicoargenteus, though a common and widespread species, is probably of little or no importance in virus transmission and no viruses have been isolated from it, though very large numbers have been inoculated into mice. It is true that specimens were included in a mixed lot of sylvan species of *Aedes* from which yellow fever was isolated in 1944 (Smithburn & Haddow, 1946) but it was concluded that it was highly probable that the infected species in the lot concerned was *A. africanus*. Twelve specimens were also included in the very large mixed lot of mosquitos from which Ntaya virus was isolated in 1943 (Smithburn & Haddow, 1951). There is, therefore, no definite evidence against *A. apicoargenteus* but it is to be remembered that although Bauer (1928) failed to transmit yellow fever in the laboratory, using this species, as did Haddow, Smithburn, Dick, Kitchen & Lumsden (1948), Woodall (1959) has recently shown that it can maintain the virus for at least 14 days and that in all probability multiplication occurs.

A. simpsoni has proved to be an important vector of human yellow fever in western Uganda, though not so far incriminated elsewhere. In 1941, two strains were isolated from lots of *A. simpsoni* collected in Bwamba County (Mahaffy & others, 1942) and a further strain was obtained in the same area in 1942 (Smithburn & Haddow, 1946). It is thought that, in forested areas like Bwamba, *A. simpsoni* plays a key rôle in picking up the virus from forest monkeys and passing it on to man (Haddow, Smithburn, Mahaffy & Bugher, 1947). In addition to the isolations from wild-caught specimens, it has been shown to be capable of transmitting the virus under laboratory conditions (Philip, 1929), and it is important to remember that *A. simpsoni*, in areas with rapid urban spread, may show a distinct tendency toward domestic or peri-domestic breeding (Muspratt, 1956). It may thus be potentially a more dangerous species in the future than it is at present. Apart from yellow fever, it may be noted that one specimen of *A. simpsoni* was present in the large mixed lot of mosquitos from which Ntaya virus was isolated (Smithburn & Haddow, 1951, see above).

A. fraseri was not included in any of the lots of mosquitos from which virus has been isolated, and no laboratory studies on its ability to transmit have been made.

Twelve examples of *A. dendrophilus* (cited as *A. de-boeri* ssp. *de-meilloni* Edw., see p. 337) were included in the mixed lot of *Aedes* from which yellow-fever virus was isolated in 1944 (Smithburn & Haddow, 1946) but, as mentioned above, it was concluded that it was very likely that the infected species was *A. africanus*. Forty were included in the large mixed lot of *Aedes* from which Bunyamwera virus was isolated but here, as already explained (Haddow, 1960), it is thought that *A. circumluteolus* was the species most likely to be involved, though of course this is merely a suggestion based on the local epidemiological picture. In 1944, however, an isolation of the virus of Rift Valley fever was made from an unmixed lot of 60 examples of *A. dendrophilus*, collected in the Semliki Forest. It is not known, however, whether this mosquito can transmit the virus by bite, as no laboratory work has yet been carried out with it.

Together with *A. circumluteolus*, *A. africanus* ranks as one of the most important virus vectors of the Ethiopian Region. The readiness with which it will bite man was first emphasised by Kerr (1933) and its ability to transmit yellow-fever virus under laboratory conditions was demonstrated by Philip (1929). In 1944, as already mentioned above, yellow-fever virus was isolated from a

mixed lot of sylvan species of *Aedes*, taken in the uninhabited Semliki rain-forest in Bwamba County, western Uganda (Smithburn & Haddow, 1946). This lot contained three examples of *A. africanus* and it was concluded that, of the various species included, *A. africanus* was the most likely to have been infected. It was also concluded, on theoretical grounds, that this species was probably the postulated vector of the monkey-to-monkey cycle of the rain-forests (Haddow, Smithburn, Mahaffy & Bugher, 1947). This view was greatly strengthened by the discovery that it is the dominant mosquito of the forest canopy in the area concerned (Haddow, Gillett & Highton, 1947). In 1947, a rhesus monkey died following an inoculation of 187 examples of *A. africanus* and one of *A. luteocephalus* from the same forest area (Haddow, Smithburn, Dick, Kitchen & Lumsden, 1948). Though this monkey, at the time of death, had developed antibodies to yellow fever, and though its liver showed histologically the picture of yellow fever with some repair, no virus was isolated, as unfortunately no material for inoculation was taken at the post-mortem examination. In 1948, however, four strains of yellow-fever virus were isolated from lots of *A. africanus* collected in the Semliki Forest, and, in the case of one lot, transmission to a rhesus monkey by bite was achieved before the mosquitoes were ground up for inoculation. The high incidence of the virus in the wild population of *A. africanus* at that time was shown by the fact that the four strains came from a total collection of 2,040 specimens—quite a small sample. It may be added that no virus was isolated from 5,258 mosquitoes of other species taken in the same catches (Smithburn, Haddow & Lumsden, 1949).

Apart from yellow fever, quite a number of other isolations of virus have been made from this species. Included in the large mixed lot of mosquitoes from which Ntaya virus was isolated were three examples of *A. africanus* (Smithburn & Haddow, 1951). In 1948, a strain of Zika virus was isolated from mosquitoes of this species collected in Zika Forest, near Entebbe (Dick, Kitchen & Haddow, 1952) and, in 1956, two further strains of this virus were isolated from *A. africanus* collected in Lunyo Forest, also near Entebbe (Weinbren & Williams, 1958). In 1955, an isolation of the atypical Lunyo strain of the virus of Rift Valley fever was made from *A. africanus* taken at Lunyo (Weinbren, Williams & Haddow, 1957), and, in 1956, a strain of chikungunya virus was isolated from a lot collected at Zika (Weinbren, Haddow & Williams, 1958). The isolations made to date from this important species are, therefore:—

Ntaya virus	1 possible?
Yellow-fever virus	4 definite, 2 presumptive
Zika virus	3
Rift-Valley-fever virus (Lunyo strain)	1
Chikungunya virus	1

A. luteocephalus is very closely allied to *A. africanus* but prefers drier country (Mattingly, 1952). It is known to be capable of transmitting yellow-fever virus under laboratory conditions (Bauer, 1928; Philip, 1930), but no virus has been definitely isolated from it in nature so far, though it is to be remembered that in 1947 a monkey died of yellow fever following an inoculation of one example of *A. luteocephalus* and 187 of *A. africanus* (see above). Lewis (1943) considered that it was probably a vector in the Nuba Mountains yellow-fever outbreak.

Several other members of this subgenus have been shown to be capable of transmitting yellow fever under laboratory conditions. These are *A. (S.) vittatus* (Big.) (Philip, 1929), *A. (S.) metallicus* (Edw.) (Lewis, Hughes & Mahaffy, 1942), *A. (S.) pseudoafricanus* (Chwatt, 1950) and *A. (S.) strelitziae* Muspratt (Gillett & Ross, 1953). It may be added that Lewis (1943) suspected that two of these species (*A. vittatus* and *A. metallicus*) were involved as vectors during the Nuba Mountains epidemic. In conclusion, it should be noted that all members of the subgenus *Stegomyia* which have so far been tested as vectors of yellow-fever virus

under laboratory conditions have proved capable of transmitting the virus by bite, with the single exception of *A. apicoargenteus*.

Summary.

The cyclic biting activities of East African mosquitos of the genus *Aedes*, belonging to the subgenera *Mucidus*, *Diceromyia*, *Finlaya* and *Stegomyia* are discussed. The total number of 24-hour catches involved was 448, all in East Africa, of which 401 were made in forest in Uganda.

It is pointed out that many species in the subgenera of *Aedes* dealt with show arboreal tendencies of varying degree and, while a species may show a preference for some particular level, many of them appear to make daily vertical migrations. Consequently, biting may reach its maximum intensity at different times at different levels. It is also pointed out that the habits of a species may vary from one area to another, and therefore it is often best to discuss a representative series of catches from a productive area, noting such differences as may have been observed elsewhere. It has been found further that, under certain circumstances, a species may fail to show a clear pattern of biting behaviour and the possible reasons for this are discussed.

All members of the subgenus *Mucidus* so far adequately studied are arboreal and nocturnal. In the subgenus *Finlaya*, *A. ingrami* Edw. is a mosquito of the forest understorey which bites most freely in the hour before sunset. It makes vertical migrations, however, and the biting cycle at ground-level differs from that in the trees. *A. longipalpis* (Grünb.) is a diurnal species of the forest canopy. A view formerly put forward by the writer, that the form of the biting cycle in this species could be attributed to different physiological groups biting at different times, can no longer be supported.

In the subgenus *Stegomyia*, *A. dendrophilus* Edw. and *A. apicoargenteus* (Theo.) are diurnal species with rather irregular biting cycles, *A. dendrophilus* preferring ground-level, *A. apicoargenteus* the understorey. The biting cycle of *A. simpsoni* (Theo.) is also diurnal and rather irregular, but usually shows its highest level in the afternoon, particularly in forest. *A. africanus* (Theo.) is a crepuscular species of the forest canopy which does, however, make vertical migrations, and which may be driven down to low levels by wind. At ground-level its biting cycle is entirely different from that obtaining above, being diurnal for the main part. *A. ruwenzori* Haddow & van Someren is also arboreal and crepuscular, but reaches its peak in the hour before sunset. Scarcer species are also discussed briefly.

Members of the ^{sub.}genus *Mucidus* are not known to be involved in disease transmission. Members of the group of *A. furcifer* (Edw.) and *A. taylori* Edw. (subgenus *Diceromyia*) are suspect as yellow-fever vectors in some areas. Either *A. longipalpis* or *A. ingrami* (subgenus *Finlaya*) was probably the species from which the original isolation of Uganda S virus was made.

It is in the subgenus *Stegomyia*, however, that the species heavily implicated in virus transmission are found—*A. aegypti* (L.) (yellow fever, dengue and chikungunya), *A. africanus* (yellow fever, chikungunya, Zika virus and Rift Valley fever), *A. simpsoni* (yellow fever) and *A. dendrophilus* (Rift Valley fever).

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THE LIFE-HISTORY AND REPRODUCTIVE POTENTIAL OF *CRYPTOLESTES PUSILLUS* (SCHÖNHERR) (COL., CUCUJIDAE) AT HIGH TEMPERATURES AND HUMIDITIES.

By K. R. ASHBY *

E.M.

*Agricultural Research Council, Pest Infestation Laboratory,
London Road, Slough.*

Cryptolestes pusillus (Schönh.) and *C. ferrugineus* (Steph.), which prior to 1939 were regarded as minor secondary pests of stored grain, became serious primary pests during the Second World War. Although infestations have since decreased in frequency with the improvement in storage conditions, these two species have remained among the most serious primary pests of stored grain in Britain and Canada, infestations caused by *C. ferrugineus* tending to be the commoner and those by *C. pusillus* the more severe (Howe & Lefkovitch, 1957).

The first major studies on the biology of the two species were by Davies (1947, 1949) on *C. pusillus* and by Rilett (1949) on *C. ferrugineus*. A similar study has since been made by Lefkovitch on *C. ugandae* Steel & Howe (1957). The work described in the present and the subsequent paper (Ashby, 1961) was done at the Pest Infestation Laboratory during 1944 and 1945 as part of its programme of research, and was designed to amplify the work which had been done by Davies four years previously. Lucas & Oxley (1946) referred to two of the conclusions, the intragranular nature of the developmental stages of *Cryptolestes* and the possible importance of cannibalism as a factor affecting the growth of populations.

Materials and methods.

The course of development of *C. pusillus* was studied at combinations of 25 and 33°C., and 70, 80 and 90 per cent. R.H., and at 37°C. and 80 per cent. R.H. Fertilised eggs for these experiments were obtained by sieving a mass culture of adults once a day. The experiments at 25 and 33°C. started with 42 eggs each, that at 37°C. with 28. The insects were reared individually in glass cells, 1 cm. in diameter and about 1 cm. deep, kept in sets of seven on three-tiered trays of wire gauze. These were supported on wire stands in 2 lb. jam jars which were sealed with snap closures. A solution of sodium hydroxide in the bottom of each jar maintained the requisite humidity. The cells were left uncovered to make the diffusion path between the NaOH solution and the medium as free as possible. There were initially 2 mg. of wholewheat flour *plus* 10 per cent. yeast in each cell, and more was added as development proceeded. Correct conditioning of the humidity of the medium before use was checked with Co(CNS)₂ papers in this and in subsequent experiments. The cultures at 25 and 33°C. were examined daily in the laboratory, that at 37°C. twice daily in a constant-temperature room maintained at 23°C., 70 per cent. R.H. Examination of the stages within the cocoon was made possible by the frequency with which larvae utilised the bottom of the glass cells to form one side of the cocoon. When this occurred, the contents could be studied with an improvised inverted microscope.

The resulting adults were used for an investigation of fecundity. As they

* Now at Zoology Department, Science Laboratories, South Road, Durham.

emerged they were paired off in glass cells, 16 mm. in diameter and about 16 mm. deep, containing about 50 mg. of the flour and yeast mixture, and the rate of oviposition was measured for four months. To prevent escape it was found necessary to cover the cells with muslin, which was kept in place by rings of cork. Otherwise the apparatus used was the same as described above. Observations were made daily until the end of the preoviposition period, and then every fourth day at 25°C. and every second at 33°C. The medium was changed at every sixth examination. Owing to the difficulty of finding eggs laid in the medium and the impossibility of sieving flour conditioned to 90 per cent. R.H., eggs were allowed to hatch, the first-instar larvae being removed. No damaged eggs or other signs of cannibalism were seen at any time (see also Ashby, 1961). A considerable proportion of the eggs was laid through the muslin on to the cork where, given the opportunity, larvae readily developed in the lenticular material. Fertile eggs were laid at a high rate at 37°C., but no detailed observations were made at that temperature.

A preliminary experiment was done to determine the effect of crowding on the oviposition rate. Adults, which had been kept unpaired at 25°C. and 70 per cent. R.H. for two months after emergence, were put into groups of two pairs each. Five of the groups were put in tubes containing 6 g. of fine flour *plus* yeast conditioned to 70 per cent. R.H., another five in tubes containing 0.12 g., and a further three sets of five groups were put on intermediate quantities. The tubes were left at 25°C. and 70 per cent. R.H. for 12 days, about twice the period normally spent in the egg, and then the contents were sieved to permit counting of eggs and larvae.

A small-scale experiment was also done to test the ability of larvae of *C. pusillus* to develop on whole grain. Twenty-one glass cells containing 1 g. of appropriately conditioned grain were put at 90, 80, 70 and 50 per cent. R.H., respectively, and an egg of *C. pusillus* was put in the crease of one of the superficial grains in each cell and incubated at 30°C. English wheat, showing some deterioration owing to storage in damp conditions, was used because of reports that sound Canadian wheat had proved an unsuitable medium. Before conditioning it contained some mites but no insects. Towards the end of the larval period the grains in half the tubes were cut open. The grains in the remainder were examined when the adults emerged.

The main experiments on the effect of crowding and all those on the growth of populations on grain were done with *C. ferrugineus* (see Ashby, 1961).

Results.

Development.

There were four instars in almost all cases, but instances of three and five were recorded. The first instar did not move much. The second and third were more active and burrowed down into the flour. Activity reached its peak near the end of the fourth instar, when the tendency to burrow disappeared, and the larvae wandered freely on the surface of the flour or even climbed on the wall of the cell with the aid of silk. The cocoon was usually substantial, but occasionally it was flimsy and pupation took place outside it. The newly formed imago spent a considerable period within the cocoon.

There were very few natural deaths, except among larvae in the culture at 25°C. and 90 per cent. R.H., where five out of a total of eight larval deaths occurred. Here the medium rapidly became a mass of moulds, whereas at 33°C. and 90 per cent. R.H., the moulds were effectively browsed. The main source of casualties was the escape of larvae during the wandering stage of the fourth instar. In addition, a third of the culture at 25°C. and 80 per cent. R.H. was inadvertently destroyed. Details of results are given in Table I and fig. 1. The figures

in brackets refer to pilot experiments where 20 mg. of food were given initially and the cultures examined only sufficiently to determine the total length of development.

TABLE I.

The pre-adult developmental period * in days of *Cryptolestes pusillus* at high temperatures and humidities.

R.H. (%)	25°C.			33°C.			37°C.		
	<i>n</i>	\bar{x}	S.D.	<i>n</i>	\bar{x}	S.D.	<i>n</i>	\bar{x}	S.D.
90	29	39	1.4	36	21	0.8			
80	19 (39)	44 42	1.5 1.5)	39	22	0.8	19	21	0.7
70	29 (29)	52 48	2.9 3.1)	31	25	1.2			

n = number of individuals which completed development.
 \bar{x} = mean duration of pre-adult development.
S.D. = standard deviation of the mean.
* Including period spent by imago within the cocoon.

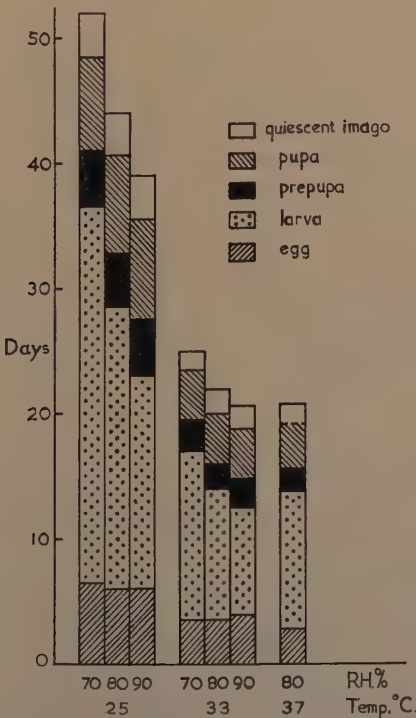


Fig. 1.—The mean duration of the developmental stages of *C. pusillus* at high temperatures and humidities (the end of the pupal stage was not accurately determined at 37°C.).

Oviposition.

At 25°C., the preoviposition period was less than six days in 18 instances and was six days or more in 17, and it tended to be rather longer than was observed by Davies (1949). At 33°C., it was less than two days in 30 cases and longer in 6. There were indications of a tendency for it to decline with increase of humidity. There was some tendency for the contacts on the thermostat of the incubator kept at 33°C. to stick and cause overheating for short periods between about the 80th and 100th day. This may have been responsible for some of the deaths at this time and possibly makes figures obtained subsequently at 33°C. less reliable than the remainder. Graphs of the mean oviposition rates are given in fig. 2. Males tended to live longer than females, most of those at 33°C. being still alive after 150 days.

At 33°C., the females laid eggs almost every day. The oviposition pattern of

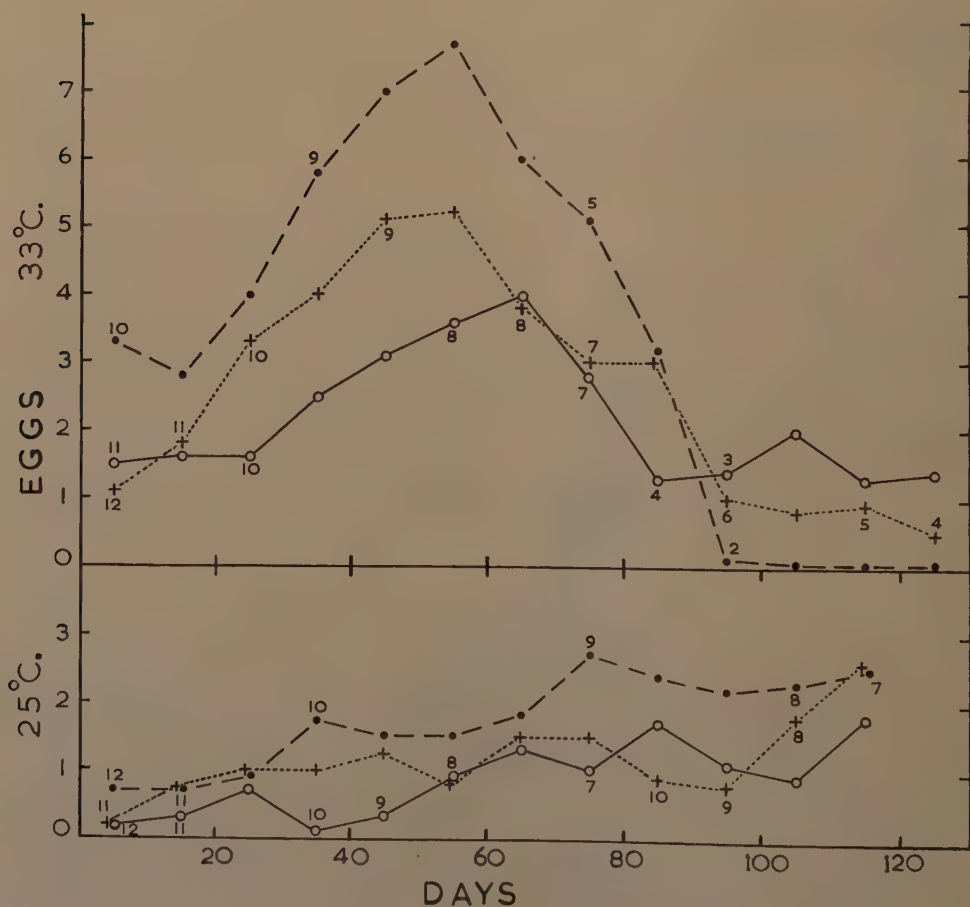


Fig. 2.—The mean oviposition rates (eggs/♀/day), plotted against age, of *C. pusillus* at high temperatures and humidities. The numbers against individual values on the curves represent the number of pairs surviving in the culture concerned.

○—○ — cultures at 70% R.H.
 +—+ — " " 80% " "
 ●—● — " " 90% " "

individual females resembled each other most at 90 per cent. R.H., where during the first 80 days the minimum number of eggs laid by any female was two-thirds of the maximum. The highest rate of laying over a short period was 121 in ten days and 42 in two days, the latter figure representing about half of the body weight of a full-sized female per day. At 25°C., while there was a rise in the average oviposition rate towards middle age similar to that seen in the cultures at 33°C., the pattern of oviposition of different females varied greatly and apparently at random. There were often considerable intervals between fertile periods when few or no eggs were laid, particularly at the lower humidities. Females that were laying ate much more than those that were not. Absence of oviposition over a long period did not necessarily indicate inherent infecundity. One female in the culture at 25°C. and 80 per cent. R.H. laid only 12 eggs during the first three months and then 109 in the following month, while another laid at nearly four times the average rate over the first three months.

The opportunity presented by the females laying eggs through muslin was used to make an approximate comparison of the weight of eggs and adults. A hundred and ten eggs laid on muslin were picked off and weighed together on a balance accurate to 0.1 mg., the most sensitive available at the time. The reading of 0.6 mg. was checked and indicated an average individual weight of about 0.0055 mg. Eggs laid in flour could not be used as it was found impossible to remove the flour sticking to the surface. Individuals of a sample of 200 adults, which was unselected and included some small individuals, had an average weight of 0.21 mg.

The experiment to determine the effect of crowding on the reproductive rate (see p. 354) gave inconclusive results owing to the big random variations in the oviposition pattern of individual females (see above) and the small numbers used. There was no indication that the differences in the number of eggs recovered were correlated with the degree of crowding in the various cultures. But while the average number of larvae (the product of oviposition in the first half of the experimental period) was similar to that of eggs in the less crowded cultures, it was distinctly lower in the more crowded. It was not possible to decide whether this resulted from a slower onset of oviposition, due perhaps to disturbance, or from a larval mortality at the higher densities. The figures indicate that it was the surface area of the flour and not its total volume that was important. The minimum depth of flour used was 0.3 cm., and it was noticed that where the flour was deeper, adults did not penetrate more than 0.5 cm. below the surface. Crombie (1944) noted similar behaviour in *Oryzaephilus*. The mean net oviposition rate during the experiment was 1.40/♀/day. This is rather greater than observed previously in females of a comparable age in the culture of individual pairs kept at 25°C. and 70 per cent. R.H. Possibly the long period of virginity influenced the result.

Culture of larvae on grain.

At the end of the small-scale experiment referred to on p. 354, the wheat at 80 and 90 per cent. R.H. was mouldy, and mites were often present. Both moulds and mites were absent at the lower humidities. There is no reason to think that the mites had affected the result. All the germs were in poor condition and at 90 per cent. R.H. they were rotten. Development was intragranular and in nearly all cases it was the endosperm that was attacked. In all but three cases, pupation occurred within the cavity that was hollowed out. The differences in humidity had a marked effect both on the proportion of cases in which development took place and the speed with which it occurred. In the 11 tubes at 90 per cent. R.H. examined after 17 days, eight larvae in the fourth instar and one pupa were recovered, whereas in the 11 tubes at 70 per cent. R.H. examined after 31 days, two larvae in the third instar and two in the fourth were found. The final figures for recoveries are shown in Table II.

TABLE II.

The fate of progeny of *Cryptolestes pusillus* from eggs placed on grains of English wheat at various humidities.

R.H. (%)	Successful development (in grain)	Died as young larvae outside grain	Not found
90	17	0	4
80	6	0	15
70	7	5	9
50	1	10	10

Discussion.

The figures for the rate of development at 25°C. recorded by Davies (1949) and the author agree except for the period spent by the imago within the cocoon. As the recorded pupal period was the same in both cases and as Davies gave no separate figure for the time spent by the imago within the cocoon, it seems that under his experimental conditions the imagines left the cocoons sooner after the end of pupation. At 33 and 37°C. the results differ considerably from those obtained by Davies at 35°C., the rate of development being greater and the mortality much lower.

The present experiments both on flour and grain support the deduction made by Lefkovitch (1957) that humidity affects *C. pusillus* much more than *C. ferrugineus* and are contrary to the conclusions reached by Bishop (1959). For instance, the experiment using grain as the medium indicates that, at 90 per cent. R.H., development, even on endosperm, is almost as quick as on flour at this humidity and that at 70 per cent. the period spent as larva is very markedly lengthened. And again, the larval period in the author's experiment on flour at 33°C. and 90 per cent. R.H. was much less than was recorded by Rilett (1949) for *C. ferrugineus* at 32.2°C.

The reactions of the two species to temperature are, on the other hand, quite similar. The relation of speed of development to temperature is almost linear between 21 and 33°C., and at about 70 per cent. R.H. the developmental periods of the two species are similar over this range of temperature (fig. 3). But *C. ferrugineus* appears more tolerant of low temperatures (see Ashby, 1961) and *C. pusillus* of very high temperatures. At 37.8°C., Rilett observed a considerable lengthening of the fourth instar in *C. ferrugineus* whereas in *C. pusillus* at 37°C. there was only a slight sign of the lengthening of the later part of the larval stage. *C. pusillus* is the more frequently found associated with hot conditions. Lefkovitch (unpublished) has evidence of the optimum temperature for *C. pusillus* being about 37°C. and of that for *C. ferrugineus* being 2°C. or so lower.

The oviposition rates recorded at 25°C. and 70 and 80 per cent. R.H. are similar to those observed by Davies at 25°C. and 75 per cent. R.H., if allowance is made for differences of presentation of the figures. Davies' figures are based on the periods when individual females were laying. If females not laying over periods of ten days or more are disregarded in the present instance, the average oviposition rate of the culture at 25°C. and 80 per cent. R.H. during the third and fourth months is raised from 1.5 to 1.9/♀/day. The data of both authors show big variations in oviposition between females, but the present experiments do not support Davies' concept of a single period of egg-laying, which could be as short as three weeks, followed by sterility. Since it is not in fact abnormal for females to start laying after a long quiescent period, Davies' conclusion from his experiment

designed to show the effect of removing males on the subsequent fecundity of the females needs revision, as he ignored females which had not oviposited while males were present but had laid after they were removed (Davies, 1947). When these females are included, the oviposition rates become 1.1 eggs/♀/day before and 0.9 after removal of males, the corresponding figures for the controls being 2.1 and 1.9. Davies and the present author also differ in their conclusions on the effect of humidity on oviposition. As reported for *C. ugandae* by Lefkovitch (1957), the

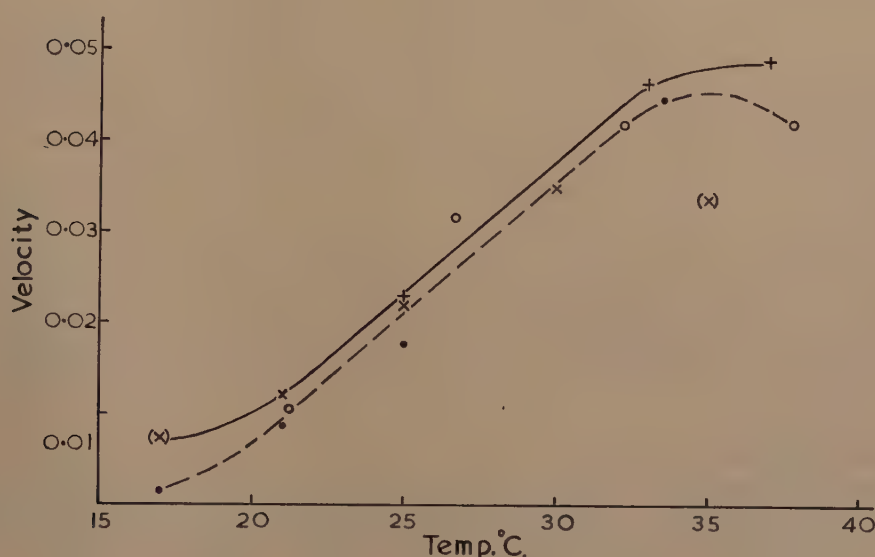


Fig. 3.—The relation between rate of development (reciprocal of time from oviposition to end of pupal stage) and temperature in *C. pusillus* on flour at 75 per cent. R.H. and *C. ferrugineus* on grain at 70–75 per cent. R.H.

x x / + + — observations on *C. pusillus* by Davies (1947, 1949)/Ashby (present work).

oo/•• — observations on *C. ferrugineus* by Rilett (1949)/Ashby (1961).

solid line/broken line — suggested relation in *C. pusillus*/*C. ferrugineus*.

The values in brackets are based on small numbers (5 or fewer). The author's figures for *C. pusillus* assume that the developmental period at 75 per cent. R.H. is the mean of that at 70 and 80 per cent., and that a rise of temperature from 33 to 37°C. reduces it to the same extent at 75 as at 80 per cent. R.H. Where a paper gives two values of similar reliability for the developmental period under given conditions, the mean of these is used.

oviposition rate in the present experiments on *C. pusillus* was much greater at high humidities, whereas Davies noted little change after transferring cultures from 75 per cent. R.H. down to 55 or up to 90 per cent. R.H. It is not clear from Davies' description that the medium was effectively conditioned to the new humidities, since he sieved the medium to remove the eggs, which was found impossible with flour at 90 per cent. R.H. in the present study. A detailed comparison with the study of Bishop (1959) on the fecundity of *C. pusillus*, *C. ferrugineus* and *C. turcicus* (Grouv.) is not possible because even under the most favourable conditions the rate of oviposition he records in *C. pusillus* is only a quarter of that found in the present experiments.

The reversal of the effect of humidity on oviposition amongst old individuals at 33°C. (fig. 2), possibly indicates that at 80 and 90 per cent. R.H. the reserves of

ova had become nearly exhausted. If this is correct, it would indicate that the maximum number of eggs that can be laid by a female is about 500.

Summary.

A study has been made of the development of *Cryptolestes pusillus* (Schönh.) at various combinations of high temperatures and humidities. Larval development is much quicker at 90 than at 70 per cent. R.H., and the optimal temperature is probably about 37°C. Under optimal conditions the developmental period is less than has so far been recorded for other species of *Cryptolestes*, the mean duration of the larval stage being as little as 8½ days at 33°C. and 90 per cent. R.H. The oviposition rate is similarly much increased at both high temperatures and high humidities. There is evidence that middle-aged females lay more rapidly than either younger or older individuals. Under optimal conditions the average oviposition rate can exceed 7 eggs/♀/day, about 17 per cent. of the weight of the adult.

In cultures of adults on flour, there was no clear indication of a reduction of the oviposition rate or of a juvenile mortality at densities ranging from 1.5 g. per adult down to 0.03 per adult, but there were signs that the latter density was near a critical value.

Eggs of *C. pusillus* were cultured on whole English grain with the germs in poor condition, at humidities ranging from 50 to 90 per cent. The endosperm rather than the germ was attacked in almost all cases. At 50 per cent. R.H., only one individual out of 21 completed its development whereas at 90 per cent. R.H. few failed to do so. Humidity had a considerable effect on the rate of development in the larval stage. At 90 per cent. R.H. it was only slightly slower on grain than on flour.

Acknowledgements.

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THE POPULATION DYNAMICS OF *CRYPTOLESTES FERRUGINEUS*
(STEPHENS) (COL., CUCUJIDAE) IN FLOUR AND ON
MANITOBA WHEAT.

By K. R. ASHBY *

E.M.N.

*Agricultural Research Council, Pest Infestation Laboratory,
London Road, Slough.*

Davies (1949) found that whereas cultures of adults of *Cryptolestes pusillus* (Schönh.) had a high fecundity on flour or crushed wheat, on sound wheat the production of larvae was negligible and the adult mortality very high. Even when slightly damaged wheat was used, few larvae survived into the later instars. Williams (1954) confirmed that newly hatched larvae are unable to enter unblemished grains, and Rilett (1949) found this was also true for larvae of *Cryptolestes ferrugineus* (Steph.).

Nevertheless, both species were reported (R. W. Howe and T. A. Oxley, personal communications) to be capable of attacking commercial grain when this was stored for a long period. The experiments described below were designed to elucidate the factors controlling the growth of populations of *C. ferrugineus*, particularly in grain.

Materials and methods.

The work is in five parts which are shown below as sections (a) to (e) and similarly marked in the "Results" (p. 365) and in the "Discussion" (p. 372).

(a) *The effect of crowding on reproduction in flour.*

The effect of crowding on oviposition, and on the survival of eggs and larvae in flour were investigated by putting adults at six different densities on a standard environment maintained at 25°C. This environment consisted of a layer, 0.5 cm. deep, of wholewheat flour plus 10 per cent. yeast, sieved to remove large particles and conditioned to 70 per cent. R.H. In five cases it was obtained by putting 7 g. of flour in a glass tube of 5 cm. diameter and in the sixth by putting 25.5 g. in a glass jar of 9.5 cm. diameter. Adults were taken from a large mass culture maintained at 25°C., derived from the 33D culture of an experiment described later (see below and p. 368), after thorough mixing to ensure uniformity. About 60 individuals were put in the jar and in one of the tubes, and 250, 500, 1,000 and 2,000, respectively, in the remaining tubes, the cultures being named from A to F in order of increasing density. The containers were covered with muslin, but no other precautions were found necessary to keep the humidity at 70 per cent. R.H. The adults could not climb the sides of the tubes and no eggs were laid on the muslin. The containers were left for 14 days and then sieved, the eggs and larvae being counted and removed. The adults were put back on fresh flour and the process was repeated. At the second sieving, dead adults were counted as well. The living adults were returned to fresh flour and the number of eggs counted after a further four days.

(b) *The history of cultures of infested grain.*

The history of cultures of *C. ferrugineus* on whole wheat at 70 per cent. R.H. was studied in conditions as closely resembling those in a grain silo as were

* Now at Zoology Department, Science Laboratories, South Road, Durham.

practicable in a small environment, changes in their composition being followed mainly by measuring the output of CO_2 (Howe & Oxley, 1944). This method avoided disturbing the cultures and the difficulties of counting intragranular stages. The cultures were kept in bottles with an air space above the grain. The output of CO_2 was determined by sealing the cultures for a known time and estimating the rate of rise in the concentration of CO_2 in the air space by analysing samples with a catharometer, readings usually being taken every second day. At other times the containers were left uncorked. The air space reduced the rate of rise in concentration after sealing and the large surface area of the grain encouraged diffusion of CO_2 from the intergranular air. Diffusion gradients were in general ignored, but at 33°C . it was found necessary to evacuate the containers and refill with air immediately prior to stoppering, to remove CO_2 which was in process of diffusing out of them.

The cultures were derived from Manitoba wheat at 70 per cent. R.H. infested with *C. ferrugineus*, received from Yarmouth. It was put at 25°C . on 28th December 1944, this being regarded as the start of the experiment. On the third day, the infested wheat was divided into 108 parts which were recombined at random to give nine cultures of very approximately 450 g. each. The adults in one of these (known as the 25T culture) were sieved off on the sixth day, and adults emerging subsequently (together with extra-granular pupae) were similarly removed after each estimation of the output of CO_2 , any larvae recovered being returned to the culture. On the fifteenth day, sufficient Canadian grain from another source at 70 per cent. R.H. was mixed gently with four of the cultures to bring their net weight up to 2 kg. These cultures are referred to as the 'D' or diluted cultures, the remaining four as 'U' or undiluted cultures. The U and three of the D cultures were transferred to quart Winchester bottles, the remaining D culture (later put at 33°C .) to an aspirator, the 25T culture being left in an 800 cc. bottle. On the eighteenth day, one U and one D culture were transferred to 17, 21 and 33°C ., respectively, the remaining pair being left at 25°C . These cultures are named by combining the number corresponding to the temperature in which they were placed at this time, and the letter U or D (e.g., 17U, 25D). Subsequent changes were followed for up to four months. The output of CO_2 was normally estimated every two days, but at longer intervals when it seemed appropriate.

(c) *Cultures on uninfested grain.*

As large infestations developed in the D cultures at 25 and 33°C ., additional cultures were set up using previously uninfested grain, and their CO_2 outputs similarly studied. Each contained 320 g. wheat at 70 per cent. R.H., and adults derived from the 33D culture were added. Other adults from the same source (cultures A and B) had already been shown to have a normal fertility. Details of the cultures are given below.

At 25°C . and 70 per cent. R.H. : (i) Control (fresh wheat), (ii) Fresh wheat + 300 individuals of *C. ferrugineus* (Culture G).

At 33°C . and 70 per cent. R.H. : (i) Control (fresh wheat), (ii) Fresh wheat + 65 individuals of *C. ferrugineus* (Culture H), (iii) Fresh wheat + 300 individuals of *C. ferrugineus* (Culture I), (iv) Wheat from 33U culture (see p. 368 and Table I) + 65 individuals of *C. ferrugineus* (Culture J).

At 33°C . and 90 per cent. R.H. : (i) Control (fresh wheat), (ii) Fresh wheat + 65 individuals of *C. ferrugineus* (Culture K).

(d) *Cultures on uninfested grain with extra food.*

Subsequently the following cultures were set up with 710 g. wheat at 70 per cent. R.H. plus a small amount of finely divided additional food consisting of 2 parts flour to 1 of yeast.

At 25°C. and 70 per cent. R.H.: (i) 600 individuals of *C. ferrugineus* which emerged in the 25T culture + 6 g. flour and yeast (Culture L), (ii) 170 individuals of *C. ferrugineus* from the 25U culture + 6 g. flour and yeast (Culture M).

At 33°C. and 70 per cent. R.H.: 300 individuals of *C. ferrugineus* from 33D culture on (i) Fresh wheat only (Culture N), (ii) Fresh wheat + 6 g. flour and yeast (Culture O), (iii) Fresh wheat + dust and broken grains from 2 kg. wheat (Culture P), (iv) 2 parts fresh wheat:1 part wheat from the 25T culture (see p. 364) + dust from 1 kg. fresh wheat (Culture Q).

A further 6 g. of flour and yeast was added to these cultures during the course of the experiment (fig. 4).

(e) *The relation between output of CO₂ and number of adults of C. ferrugineus.*

During the various experiments, weights and CO₂ outputs of known numbers of adults were measured when possible, and samples of fourth-instar larvae were also weighed. The CO₂ outputs of three cultures of larvae on flour at 33°C. and 70 per cent. R.H. (R, S and T) were determined in a separate experiment. Initially all the larvae in R were in the fourth instar, those in S in the third and those in T in the second.

Results.

(a) *The effect of crowding on reproduction in flour.*

The results are summarised in fig. 1. In A and B there was no indication that there had been an appreciable mortality amongst eggs or larvae or that the difference of density affected the rate of oviposition, and their numbers were a direct function of the number of adults present. The average fecundity was 1.28 eggs/♀/day. The proportion of eggs to larvae after each period of 14 days indicates an incubation period of about 6.2 days.

In the remaining cultures, the disproportionate reduction in the number of larvae indicates a high mortality, and there was also direct evidence of cannibalism. After 14 days, several mangled larvae were recovered from C and D (38 dead compared with 16 living at the second sieving in D), and a few in E, while after four days several damaged eggs were recovered from E and F. The total of living larvae found in the latter two cultures was only 14 in the whole experiment. The mortality of adults in four weeks was between 12 and 16 per cent. in cultures C to F and did not increase with density. After each period of 14 days, the medium in E and F was denuded of its finer particles, the material remaining being coarse particles of endosperm, but the appearance of the flour was not changed after four days.

(b) *The history of cultures of infested grain.*

The more important results are illustrated in figs. 2 and 3. The outputs of CO₂ are expressed as percentage per day of the intergranular air present after the grain was added to the D cultures. The volumes of intergranular air were calculated indirectly from the following observations made on some of the grain used for dilution:—

$$\frac{\text{Volume of intergranular air (cc.)}}{\text{Weight of grain (g.)}} = 0.467$$

Control at 25°C. (culture 25T).—Results obtained from this culture are shown in fig. 2. They demonstrate the intragranular development of a population of pre-adult stages of *Cryptolestes* which was fairly uniform in age-structure, during the first two months of the experiment. A few young larvae were seen on sieving 11 days after the experiment started and some rather more advanced individuals a week later. Larvae were seen in large numbers at the end of the fourth instar

when they wandered, presumably in search of a place to pupate, or at least readily emerged from the grains when disturbed. They practically all returned to the grains to pupate. The mean date for the formation of extragranular cocoons was 2 days after that for the wandering stage, and the mean date for emergence of

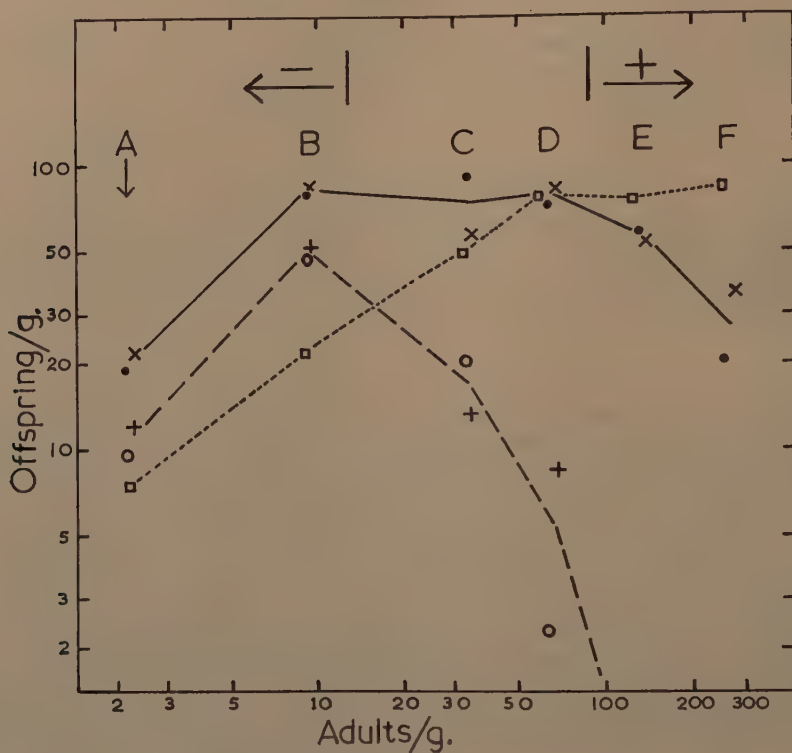


Fig. 1.—The effect of density of adults of *C. ferrugineus* cultured on flour on that of eggs and larvae.

- × × / • • — eggs plus larvae after first/second fortnight
- + + / ∞ — larvae (only) " " " "
- — — connects mean values for eggs plus larvae
- — — — □ — eggs " " " " larvae (only)
- A—F — eggs after final four days
- — — — — designation of culture
- ↑ — — — — — no juvenile mortality
- ↓ — — — — — negligible survival of larvae

adults was 17 days after that for pupation. Adults recovered before and during the peak of emergence averaged between 0.23 and 0.26 mg. in weight, those emerging subsequently below 0.20 mg. The only animals recovered at the initial sieving besides *C. ferrugineus* were a moderate number of individuals of *Oryzaephilus surinamensis* (L.), mostly dead. Later, six adults of *Sitophilus granarius* (L.) and two of *Oryzaephilus* emerged.

The output of CO₂ rose only slowly whilst the culture consisted of growing larvae, and in fact there was a net fall in output between the 21st and 35th day (fig. 2), indicating a heavy mortality during this period. Comparison with cultures

which were left undisturbed suggests that sieving was a probable cause of this mortality in spite of development being intragranular and the larvae apparently protected against direct damage. Possibly the disturbance resulted in an unusual tendency for the larvae to leave their feeding places for some time afterwards, and that those that did so usually failed to find another suitable site. Once feeding ceased, this effect disappeared and the fall in output of CO_2 at the onset of pupation was of the expected proportions.

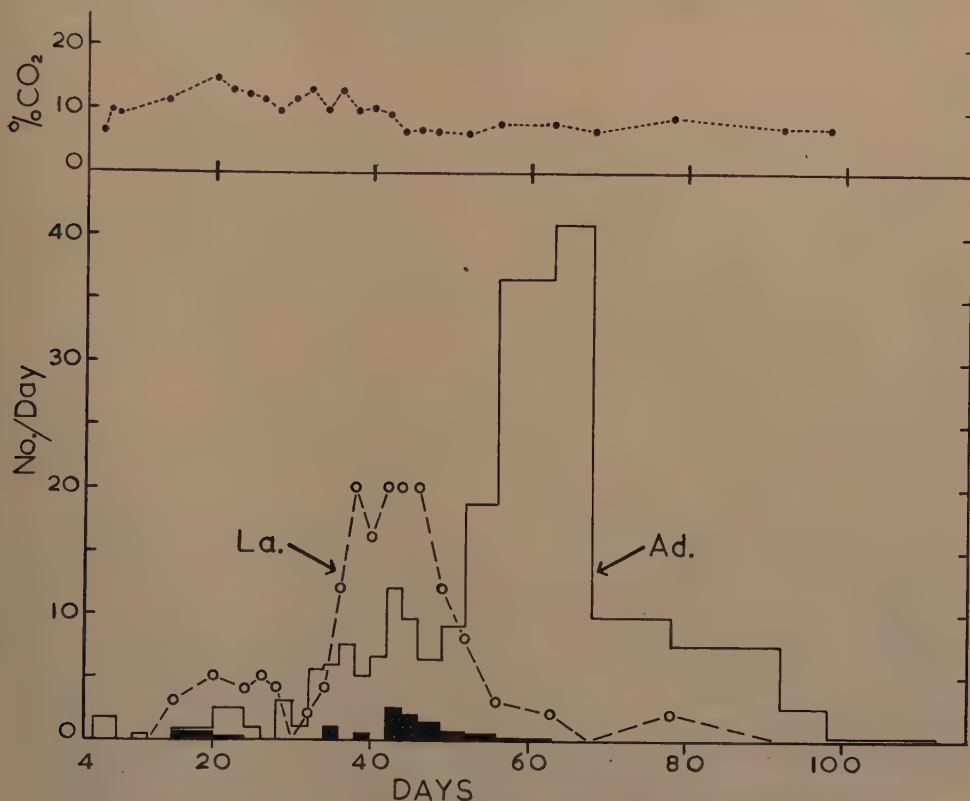


Fig. 2.—The production of CO_2 (% intergranular air/day) including that of adults already removed, and the results of breeding out in culture 25T (weight 490 g.). Recoveries of fourth-instar larvae are shown by the curve marked La. Pupae sieved off are shown in solid black, and the rate of emergence of adults is indicated by line marked Ad.

Experimental cultures at 25°C. (cultures 25U and 25D).—Initially there was a rapid rise in the output of CO_2 in both the experimental cultures at 25°C. In the U culture the gradient of the curve became slight after 20 days, and a peak of 60 per cent. CO_2 per day was reached after 30 days. This was followed by a sharp fall. Subsequent events were out of keeping with those in the other U cultures, and on sieving after 94 days it was found that a large population of *Sitophilus* had become established and that only 200 living individuals of *Cryptolestes* remained. In the D culture, the rate of rise in the output of CO_2 remained high up to the 31st day, and a peak was reached after about 37 days (fig. 3). The interruption in this rise, which occurred between the 21st and 25th day, followed the sieving of part of the culture in an attempt to get an estimate of the CO_2 output of a given number of adults. After the peak there was a decline for about a week, which coincided

with the fall in output of CO_2 associated with the onset of pupation in the 25T culture, and then a fairly slow rise in output up to a second peak reached after 92 days (fig. 3). It appears, like the first peak, to have been due to the development of a population of *Cryptolestes* larvae. After the second peak, the culture was sieved as indicated in fig. 3. The decline in the output of CO_2 continued unchecked after the cessation of sieving and does not seem to have been primarily caused by it. A total of only 132 individuals of *Sitophilus* was recovered, whereas at the early sievings many larvae of *Cryptolestes* in the fourth instar were seen. There were no signs of *Sitophilus* being present in any cultures except those at 25°C.

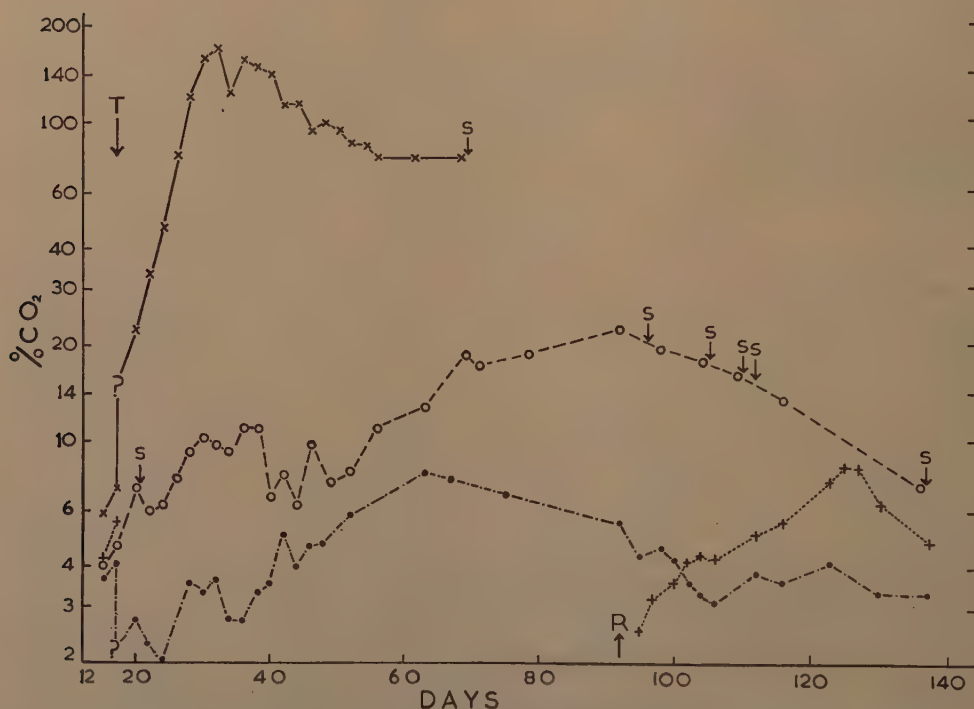


Fig. 3.—The production of CO_2 (% intergranular air/day) in the D cultures (each weighed 2 kg.): x—x in 33D, o—o in 25D, ●—● in 21D, +----+ in 17D (readings while at 17°C. too low to be included).

- T — date of transfer of cultures to respective temperatures
- ? — estimated output immediately after transfer
- R — date of transfer of 17D from 17 to 21°C.
- S — culture sieved

Cultures at 33°C. (cultures 33U and 33D).—Following the rise in output of CO_2 resulting from the rise of temperature, there was a further increase in the U culture which was at first very rapid but soon became slight, up to a peak of 200 per cent. per day reached 15 days after the transfer to 33°C. It then fell rapidly for ten days to 0.4 of the peak value, and then more gradually. On sieving after 71 days, 930 living adults, 1 larva and a much larger number of dead adults were recovered. Larvae and pupae remaining in the grains were producing only a small amount of CO_2 . In the D culture, the rate of rise in output after the transfer to 33°C. remained very rapid for 13 days and the fall subsequent to the peak was gentle (fig. 3). The peak outputs were reached simultaneously in the

two cultures. During the peak and for a few days afterwards, many wandering fourth-instar larvae were seen in the D culture, and as the output of CO_2 decreased they were replaced by still larger numbers of adults. A similar phenomenon on a smaller scale was noted in the 25D culture. On sieving after 71 days, nearly 16,000 living adults, relatively few dead adults and 100 fourth-instar larvae were recovered. The larvae and pupae remaining were producing about 15 per cent. CO_2 per day. Just before sieving, the temperature at the centre of the grain was 1°C . above that peripherally. A summary of the final condition of the grain is given in Table I. Much of the fresh grain added to the 33D culture must have been attacked during the course of the experiment.

TABLE I.

Final condition of grain in cultures of infested wheat transferred to 33°C .

Culture	R.H. of grain (%)	Loss in wt. (%)	Wt. of frass (% that of grain)	Size of random sample*	Damage to grain (%)			
					Germ† only	Endo-sperm only	Germ† and endo-sperm	Not damaged
33U	70	5.5	0.7	136 grains	72	0	14	14
33D	80	4.7	1.1	173 „	36	2	36	25

* Taken after mixing the grains.

† Where attacked, the germ had in all cases been completely eaten.

Cultures at 21°C . (cultures 21U and 21D).—Following the change of temperature, the production of CO_2 fell to rather under half its previous value in the U culture and rather over half in the D culture. In the U culture, it remained approximately constant for 100 days, then fell rapidly. On sieving, the majority of adults were found to be dead. In the D culture it rose to a peak, reached rather more than 40 days after the transfer to 21°C ., then fell slowly for about a further 40 days, after which it remained approximately constant (fig. 3). The initial fluctuations which occurred in both cultures after the transfer from 25° to 21°C . resulted from faulty temperature regulation of the incubator. The equivalent part of the 17D curve (see below and fig. 3) was much smoother.

Cultures at 17°C . (cultures 17U and 17D).—The output of CO_2 fell in both cultures at 17°C . after the change of temperature, initially to rather below a third of that at 25°C ., and six weeks later to about a sixth (this section of the curve in the D culture is omitted from fig. 3). After ten weeks at 17°C ., the approach of spring prevented this temperature being maintained and the cultures were transferred to 21°C . Events in the D culture then substantially repeated what had already occurred in the 21D culture (see fig. 3). The decline in the output of CO_2 after the peak may have been steeper but the culture was not kept long enough to confirm this. The history of the 17U culture resembled that of the 17D culture, but the increase in output of CO_2 was smaller and the peak was reached a day or two earlier.

(c) *Cultures on uninfested grain.*

No infestation developed in any of the cultures on fresh wheat at 70 per cent. R.H. (cultures G, H and I), and at 33°C . a heavy mortality of adults commenced after three or four weeks. In culture J, only six adults were still alive after the

latter period. In cultures of adults from the same source on flour, the death-rate did not exceed 16 per cent. during the same period of four weeks (cultures C-F, see p. 365).

An infestation did develop in culture K. Here the R.H. was initially 90 per cent., but was allowed to fall to 80 per cent. after five days to try to check the production of CO_2 by moulds. It was still 80 per cent. three weeks later. During this period, a generation of *Cryptolestes* developed in the creases of the grains. The wandering stage of the fourth instar was first noticed after 20 days, as in the cultures at 70 per cent. R.H. Six days later there was a mixture of fully developed larvae, prepupae and pupae. After five weeks, there were signs that a second generation was developing, this time within the grains. The output of CO_2 rose rapidly while at the same time the amount of mould declined, presumably owing to its being eaten.

(d) *Cultures on uninfested grain with extra food.*

Neither of the cultures at 25°C. (cultures L and M) became established. The same applied to the cultures at 33°C., N and O, where no damaged grains were added (fig. 4). In culture N, with no extra food initially, the adults soon started to starve, and the addition of extra food after three weeks only checked the decline in CO_2 output for a few days. In culture O, the onset of food shortage was delayed but the general trend was similar. There was no reason to anticipate a heavy natural mortality during the experiment as the insects were about 55 days old initially, 35 days of this period having been spent at 25°C.

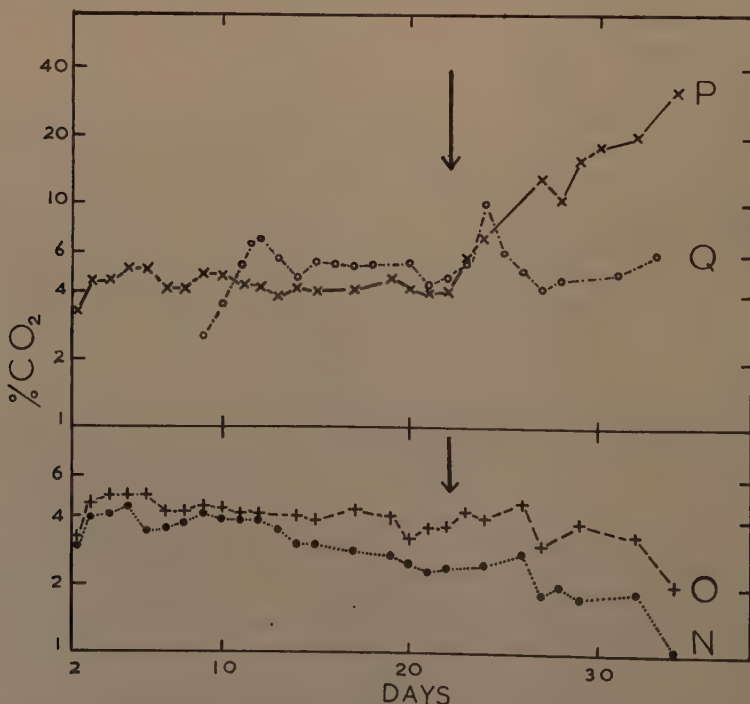


Fig. 4.—The production of CO_2 (% intergranular air/day) in four cultures of *C. ferrugineus* on grain at 33°C. to which 6 g. of flour *plus* yeast was added initially (except in N) and again during the experiment. The date of the latter addition is shown by the arrows. Culture Q was set up 8 days after N, O and P.

In culture P, there was no shortage of food at the end of three weeks, but no infestation had developed, although one larva was observed to develop in the crease of a grain, the adult emerging after 21 days, and a few other larvae may have been present. On the addition of 6 g. of flour and yeast at the end of three weeks, an infestation was immediately initiated. The output of CO_2 rose rapidly, and 15 days after adding the flour a large number of pupae and one newly emerged adult were seen in creases of the grains. In culture Q, the initial rise and fall in the output of CO_2 indicated that an infestation was initiated but that it failed to become established. This occurred again on a larger scale following the addition of flour after two weeks, but the moderate rise which became apparent a week later perhaps indicated that on this occasion some larvae had survived.

(e) *The relation between output of CO_2 and number of C. ferrugineus.*

Adults.—Reliable estimates were found to be difficult to obtain because:—

(1) Adults of *Cryptolestes* in the fairly high densities required to give easily measured outputs soon starved on sound grain alone, while oviposition could lead to the development of embryos and larvae and raise the output of CO_2 by an unknown factor if extra food was added.

(2) In cultures where there was no large air space above the grain to reduce the rise in concentration of CO_2 while the containers were sealed, the rate of release of CO_2 was unduly low for the first 24 hours or so after a culture was set up, perhaps owing to CO_2 being absorbed by moisture in the grain until an equilibrium was reached. This period is a significant part of the time taken for eggs to hatch at the higher temperatures.

(3) At the higher temperatures, established cultures could be used only if the number of adults greatly exceeded that of pre-adults, owing to the rapidity with which the CO_2 output of the juvenile stages changed under these conditions.

Estimates that appeared to avoid these difficulties are shown in Table II. The

TABLE II.

The production of CO_2 by adults of *C. ferrugineus* living on grain at 70% R.H.

Temperature and culture (1)	Output of CO_2 (2) (% intergran. air/day)	Mean wt. (adults)	No./lb. grain giving 1%/day	Production of CO_2 (cu.mm./g. insect/hr.)
33°C. 33D I N, O, P, Q	61.7 9.5 min. 3.8 (3) max. 4.8 (3)	0.24 mg. 0.25 mg. 0.24 \pm 0.01 mg.	58(5) 46 50 40	6,400 (5) 8,000 7,300 9,200
25°C. G L	4.0 \pm 0.4% 3.0 \pm 0.4%	0.25 mg. 0.20 mg.	106 132	3,300 3,400
21°C. 21D, 21U 17D, 17U	Mean drop in output to $\frac{1}{2}$ on transfer 25 \rightarrow 21°C. (4)		200 to 250	c. 1,700
17°C. 17D and 17U	Initial drop to $\frac{1}{2}$ Later drop to $\frac{1}{4}$	} on transfer 25 \rightarrow 17°C.		350 \rightarrow 700
				1,000 \rightarrow 600

(1) The constitution of these cultures is given elsewhere.

(2) Making allowance for output of wheat alone (and pre-adults in 33D).

(3) Based, respectively, on 4% and 5%, being values at which the production remained steady for some days (see fig. 4).

(4) Allowance made as far as possible for the elimination of larvae in 21U and their continued development in 21D after change of temperature (see p. 374).

(5) See p. 377.

production of CO_2 varied with factors other than temperature, even when there was no evidence of the development of larvae on the one hand or the onset of starvation on the other.

Larvae.—Two large samples of fully developed fourth-instar larvae reared in flour were weighed. The mean individual weight was 0.45 mg. and 0.46 mg., respectively.

The cultures of larvae on flour used for estimating their production of CO_2 (cultures R–T) did not give completely reliable results, as the larvae failed to pupate and as the evacuation of the cultures prior to making estimates of the output of CO_2 caused the R.H. of the flour to fall to 57 per cent. in the course of a fortnight.

The failure to pupate and the ultimate death of the larvae seem to have resulted from dietary deficiency since there was no sign of disease in any other culture. The flour on which the larvae were reared had been sieved many times, and Williams (1954) reports a very high mortality at the time of pupation when *C. pusillus* is reared on flour with an extraction rate of 85 per cent. or below.

While the cultures appeared healthy, their CO_2 output from the second to early fourth instar increased exponentially with a gradient of $\times 10$ in 8 or 9 days. The maximum CO_2 output reached would indicate that, at 33°C., about 33 larvae in the fourth instar are needed to give 1 per cent. CO_2 /lb. grain per day, the production of CO_2 being 6,100 cu.mm./g. insect/hr. assuming a mean individual weight of 0.45 mg.

Discussion.

(a) In the experiment on the effect of density on reproduction, the fecundity of *C. ferrugineus* at 25°C. and 70 per cent. R.H. observed in cultures A and B is similar to that observed in *C. pusillus* of a comparable age under these conditions (see Ashby, 1961). The calculated incubation period is also similar to that in *C. pusillus* at 25°C., being a little over six days in each case.

The effect of crowding on the reproduction of insects, and particularly of *Tribolium* and *Oryzaephilus* living in flour or grain, has already been extensively studied (Crombie, 1942, 1944; Boyce, 1946 and Andrewartha & Birch, 1954). In these genera, the reduction in the net rate of reproduction at high densities was due to a juvenile mortality and a reduced rate of oviposition, the only important cause of the former being cannibalism, the intensity of which was directly proportional to the density of adults. The reduction in the rate of oviposition was attributed to disturbance of ovipositing females. When conditioning of the medium was not significant, the operation of these two factors led in cultures of *Tribolium* to the development of an equilibrium between oviposition and cannibalism with the density of eggs at equilibrium decreasing with increasing density of adults (Boyce, 1946).

In cultures D, E and F of the present experiment, the number of eggs recovered after four days was independent of the number of adults (fig. 1). A possible explanation of this is that mortality here also was occurring at a rate directly proportional to the number of adults (with the probability of an egg surviving as long as four days small), but, in contrast with the situation in *Tribolium* and *Oryzaephilus*, the rate of oviposition being unaffected by density. After 14 days, it is possible that there had been time for such an equilibrium between oviposition and mortality to be reached in C also, as the mean number of survivors in C and D after the longer period was similar to that in D, E and F after four days. The densities used by the author are similar to those studied by Crombie, his maximum being 160 individuals of *Oryzaephilus* per gramme, the adults of which are about twice the weight of *Cryptolestes*.

The decline in the number of survivors in E and F after 14 days is associated with the conditioning of the medium and could have resulted from a reduced rate of oviposition or more intense cannibalism, or both. The latter could have

resulted from greater activity of the adults either because they were actively seeking eggs or because with less adequate food available in the flour they stayed feeding at individual spots for shorter periods, or from eggs being more readily discovered in flour with the finer particles removed. Depression of the oviposition rate as a result of conditioning of the medium is already well documented (Crombie, 1942, 1944). Recovery from conditioning was evidently complete within four days. Crombie (1944) reported a similar rate of recovery. The inversely linear relation of the densities of adults compared with the mean densities of eggs recovered after 14 days in E and F, is suggestive of the convex portion of a logistic curve of growth of population.

Although the number of surviving offspring in B after 14 days was about the same as in C and D, this did not appear to result from a state of equilibrium having been reached in B. Lefkovitch (1957) similarly found no cannibalism among larvae of *C. ugandae* Steel & Howe at low densities. It is possible that initially there was a dietary constituent present that was eaten in preference to eggs and larvae and was soon exhausted at all but the lowest densities, this occurring long before the medium as a whole became significantly conditioned. The medium had been sieved several times through silk of 40 meshes to 1 cm., which would have tended to remove bran, germ and yeast to a greater extent than endosperm. In the experiment to determine the oviposition rate of individual pairs of *C. pusillus*, where the medium was unsieved wholewheat flour plus yeast (Ashby, 1961), there was no sign of cannibalism even though the amount of food per adult was rather less than in culture C, and the medium changed less often. Williams (1954) observed more cannibalism among larvae of *C. pusillus* reared on white flour than in cultures where they were feeding on wholemeal flour or grain. If this hypothesis is correct, *C. ferrugineus* will be intermediate between *Rhyzopertha*, which is cannibalistic only in the total absence of food (Crombie, 1942), and *Tribolium* and *Oryzaephilus*, which are cannibalistic under all conditions (Crombie, 1942, 1944).

Whatever may be the detailed interpretation of the results, the experiment shows that heavy losses of eggs and first-instar larvae are to be expected if they are exposed to high densities of adults, and that the mortality will probably be increased if the latter have little readily available food, as appears to be the case with *C. ferrugineus* on sound grain at 70 per cent. R.H. Older larvae are likely to be more or less immune because of reduced vulnerability and the fact that they are normally intragranular. They are themselves likely to be predatory as has been shown in *Tribolium*, *Oryzaephilus* and *C. ugandae*, if they come in contact with eggs, young larvae or pupae. Their net effect may be greater than that of adults since they do not contribute to the stock of eggs. The frequency of cannibalism by larvae would be increased if there is any tendency for eggs to be laid near or inside cavities occupied by larvae or for newly hatched larvae to enter these cavities.

(b) The experiments with cultures on whole grain with a proportion of grains already damaged show that infestations can be initiated on this medium at 70 per cent. R.H. The maximum densities reached were above those so far recorded in the field, where the highest published figures are about 4,000 adults plus larvae per kg. (Lucas & Oxley, 1946; Finlayson, 1950). In the 25T culture, the sharpness and symmetry of the peak of emergence of adults (fig. 2) indicates that nearly all the eggs from which these had developed were laid over a short period. A comparison with Rilett's (1949) figures for pre-adult development and the evidence of the presence of young larvae 11 days after the experiment started, suggest that this period was shortly before the initial sieving. Errors in calculation resulting from the presence of individuals resulting from eggs laid at an earlier period were minimised by ignoring all stages recovered before the 31st day together with larvae recovered after the 69th day. In the remaining cultures, the CO₂ curves indicate the presence of similar populations of juvenile *Cryptolestes* while they

were at 25°C., which continued to develop after the transfer to other temperatures except in the 21U culture, where the larvae seem to have been quickly eliminated, and possibly in the cultures at 33°C. where changes in their output of CO₂ were masked by those resulting from larvae formed subsequently to the rise in temperature. The wandering phase observed at the end of the fourth instar may be general in *Cryptolestes*, there also being evidence of one in *C. pusillus* (Finlayson, 1950; Ashby, 1961) and in *C. pusilloides* (Steel & Howe) (Lucas & Oxley, 1946).

In the cultures transferred to 33°C., a second and larger population of larvae than that referred to above resulted from oviposition soon after the transfer. But in the 33U culture the great diminution of the gradient of increase of production of CO₂ after a few days indicates (as in the 25U culture) a subsequent heavy larval mortality which continued amongst the adults after pupation was completed. Culture J confirmed that the grain of the 33U culture could not continue to maintain *Cryptolestes*, whereas at the end of the experiment the grain in the 33D culture, where the R.H. had risen to 80 per cent., was maintaining a very dense population. Two kilogrammes of wheat will contain about 54,000 grains (Howe & Oxley, 1944), and, judging from the figures in Table I, there will have been about 40,000 damaged grains, so that terminally there was a proportion of two adults to five damaged grains in 33D. It would seem that, at 70 per cent. R.H., only the germs of Canadian wheat can be attacked to any extent by *C. ferrugineus*, but that if sufficient metabolic water accumulates, the endosperm becomes available as well (Table I).

In the 25D culture, there is no evidence of a second period of larval infestation having immediately followed the addition of more grain, and the broad second peak seems to have resulted from a long period of moderately successful oviposition which started while the insects which had given the first peak were pupating, and continued until large larvae from this second period of successful oviposition developed in large numbers. The developmental history of this culture apparently lends additional support to the view that large larvae can reduce the degree of survival of the young stages, as there is no reason to think that egg-laying ceased throughout the interval between the first and second periods of successful oviposition.

In the cultures transferred to 21°C., the lower temperature was associated with the subsequent absence of successful oviposition, and, in the 21U culture, with the virtual elimination of the pre-existing young larvae. It would appear probable that from 33 down to 21°C., the effect of the decreasing oviposition rate, and the increasing period of vulnerability to cannibalism resulting from slower development, exceeded that of any reduction in the predatory tendencies of the adults. The greater survival of larvae in the 17U than in the 21U culture possibly indicates that between 21 and 17°C. the intensity of predation dropped proportionately more than the further slowing of development, so that, by the time the temperature of the 17U culture was raised from 17 to 21°C., the larvae were safely within the grains. The results do not indicate whether or not oviposition is more likely to outstrip the juvenile mortality at 17 than at 21°C.

Comparable results to those obtained in this experiment were obtained by Davies (1949) with his cultures of *C. pusillus* on crushed grain at 25°C. and 75 per cent. R.H. In his two cultures with the highest initial populations of adults, the curve of heat output was similar to that of the CO₂ output in the 33D culture. It was approximately exponential from about the 15th day until the peak was reached after 32 days. The latter period is within the range of experimental error for the developmental period of *C. pusillus* up to the middle of the fourth instar, as determined by Davies and by Ashby (1961). The peaks in these cultures were proportionately narrower than in the 33D culture, indicating that the larval population was more homogeneous and that probably the initial period of successful oviposition was relatively shorter. In his two least crowded cultures, on the

contrary, Davies observed only a very slow decline from the peak value, so that here it seems that successful oviposition continued on a reduced scale for a considerable time.

The restriction of successful oviposition to only a short period after adults gain access to a new source of food or following a change to more nearly optimal conditions would seem to be a common occurrence in *Cryptolestes*. One factor likely to be involved in cultures on grain is the shortage of food readily available to *Cryptolestes* in the adult stage. As it becomes difficult to obtain, cannibalism will intensify relatively to oviposition. Once established, a colony of larvae may themselves inhibit further production of larvae by simple competition as well as by cannibalism, since they are likely often to pre-empt the most favourable microhabitats, in this case cracks giving access to the germs. The more intense and successful the initial oviposition, the greater the subsequent inhibition is likely to be. This may account for the period of successful oviposition after diluting the 33D culture being relatively shorter than that in the 25D culture. Lefkovitch (personal communication) further suggests that, as has been found in a study of *Calliphora*, a relatively high concentration of newly emerged larvae is more likely to survive and develop than are a few scattered individuals. Perhaps several larvae can together bore into a grain where one alone would fail, as has already been demonstrated for adults of *Rhyzopertha* and *Tribolium* (Andrewartha & Birch, 1954, p. 346). If this factor is important, it will accentuate the effect of the previously mentioned factors.

In cases where there is a short period of successful oviposition leading to the production of a large number of larvae, and no evidence of heavy larval mortality, it is possible, assuming that temperature affects different stages similarly, to obtain estimates of the duration of development at different temperatures from observations on the length of time taken to reach the maximum output of CO₂ or heat. Where the output of the adults originally present is relatively small it is possible also to check these estimates by comparing the gradients leading to the peak.

The following data can be used as the basis for such estimates:—

Developmental period at 25°C.: the period up to the mean date for emergence of adults in the 25T culture less half the time up to the initial sieving.

Comparison of rates of development at:—

25°C. and 33°C.: the initial peak in 25D was 37 days after the start of the experiment and the peak in 33D was 15 days after the transfer to 33°C.

25°C. and 21°C.: the peak in 21D was about 44 days after the transfer to 21°C. (judged by eye in conjunction with data given by 17D when at 21°C.), the comparable period in 25D being 20 days.

21°C. and 17°C.: culture 17D, having been 72 days at 17°C., then took about 11 fewer days at 21°C. to reach the peak than did 21D.

The maximum gradients were as follows:

At 33°C., $\times 10$ in about 11 days in 33D.

At 25°C., $\times 10$ in about 25 days (estimate based on 16th to 31st day in 25D omitting period immediately after sieving, and supported by 16th–18th day in the other seven experimental cultures).

At 21°C., $\times 10$ in about 60 days (estimate based on curves of both 21D and 17D).

The results of calculations based on these criteria are shown in Table III. estimates based on gradients being in brackets. The latter must be regarded as only approximate owing to the presence of a substantial number of adults in the cultures, but they confirm that the criteria used in the main estimates are sound. The data deduced from the 25D culture are also supported by the curves in Davies' cultures where both the speed of larval development and the rate of rise of output of CO₂ were rather greater than in the 25D culture.

These figures obtained indirectly are in quite close agreement with those obtained from direct observation by Rilett (1949) at 75 per cent. R.H. The standard deviation for the date of emergence in the 25T culture was 13 days so that the differences are well within the range of experimental error. This indicates on the one hand the reliability of the indirect method of estimation over the range of temperature used and on the other, that the degree of interference inherent in making direct observations need not cause appreciable alteration in the speed of development. In making a comparison, the time spent as a quiescent imago

TABLE III.

Estimates of pre-adult developmental period⁽¹⁾
of *C. ferrugineus* in grain at 70+ % R.H.

Temp (°C.)	Developmental period
33+ ⁽²⁾	24 (26) days
25	60 "
21	130 (140) "
17	c. 850 "

(1) Including time spent as quiescent imago.

(2) See text, below, for estimated temperature correction.

before emerging should be deducted from the author's figures as Rilett, like Davies (see Ashby, 1961) does not allow for it. The results in the 25T culture show that, at 25°C., *C. ferrugineus* spends about the same time within the cocoon as *C. pusillus*, while Rilett's figures show that the pupal period is also about the same in the two species. Probably the imago remains quiescent for a similar time as in *C. pusillus* (which is 3½ days at 25°C. and about half this period at 33°C.). A correction should also be made for the rise of temperature in the 33D culture due to metabolic activity. Assuming that the increase at different stages was proportional to the output of CO₂ (fig. 3) and that the mean rise of temperature of the culture (which was disc-shaped) was half that at its centre, an addition of 0.5°C. to the stated culturing temperature is perhaps appropriate.

The figures indicate that, at 17°C., development is very slow indeed, but there was no sign of an increased mortality like that observed by Davies in *C. pusillus* and by Lefkovitch in *C. ugandae*. This accords with reports that *C. ferrugineus* is more tolerant of cold than the latter two species (Howe & Lefkovitch, 1957).

(c) The developmental history of cultures of adults on commercial grain at 70 per cent. R.H. confirms previous observations by Rilett, Davies and Williams, that infestations of *Cryptolestes* will not develop on it under normal experimental conditions because the adults cannot penetrate the testa and therefore starve. But Lucas & Oxley (1946) have reported that, on commercial grain in bulk, *C. pusilloides* can probably pass through several generations at low densities, which varied in their observations between 5 and 10 adults per kg. Perhaps under these conditions there are sufficient damaged grains present to support the small number of adults, while cannibalism is reduced in intensity because the chance of eggs and young larvae being discovered is relatively small. Experiments at such densities with a large bulk of grain have not been attempted in the laboratory.

(d) The developmental history of cultures on commercial grain with additional readily available food show that a point was suddenly reached when a large-scale infestation will start, P providing an example of an infestation that was probably

just successful, and Q of an initial infestation that failed and a later one that was just on the border line between failure and success, with a few larvae developing far enough to be immune from predation. Rilett (1949) reported the mean period from oviposition to the onset of pupation to be 20.5 days at 32.2°C. and 75 per cent. R.H. The precocity of the formation of pupae after the addition of extra food in P (p. 371) shows that the infestation was not due primarily to a subsequent increase in the oviposition rate, but to the food causing the survival of offspring from eggs already laid. This could have been due to a reduction in the amount of cannibalism by the adults or to the provision of a finely divided extragranular source of food for the young larvae, or to a combination of the two.

Similarly, the success of the infestation in culture K (p. 370) may have resulted primarily from the presence of an extragranular source of food, provided in this case by the moulds which developed. The large number of larvae which developed outside the grains initially indicates that this was an important factor. Humidity may, in addition, have had a direct effect, since Holdaway (1932) showed that cannibalism by *Tribolium* is more intense in a dry than in a damp medium. Smallman (1944) reports that, in Canada, major infestations of *C. ferrugineus* were very strongly correlated with the presence of patches of "tough" grain. If germination starts, the testa will be ruptured and *Cryptolestes* will have ready access to food.

(e) Of the measurements of the output of CO₂ in relation to number and weight of adults (Table II) at 33°C., those giving the lower values for the respiration rate are probably the more reliable. The figures for the 33D culture are based on a very large number of individuals with a stable output of CO₂, and the error of calculation resulting from consumption of grain by *Cryptolestes* during the experiment is unlikely to be large. It would require 27 per cent. by volume of the grain to have been replaced by air for the corrected figure for number of adults producing 1 per cent. CO₂ per lb. of sound grain per day to become 50 instead of 58. The figures in Table I indicate that this would be an overestimate of consumption. The maximum values for respiration rate obtained in cultures O, P and Q were probably inflated by the presence of developing embryos, although differences in the proportion of protein in the diet may have been partly responsible for the variations observed (see Wigglesworth, 1953, p. 413). If the above interpretation is correct, the respiration rate, like the velocity of development, will vary linearly with temperature over the range 21 to 33°C. This, together with the greater success of cultures at the latter temperature, indicates a high optimum temperature in *C. ferrugineus*, as in *C. pusillus* (see Ashby, 1961).

The production of CO₂ per individual at 25°C. is about twice as great as was reported by Howe & Oxley (1944). Their technique of airing the grain prior to making estimates may release CO₂ dissolved in moisture in the grains, so making subsequent readings comparable to the low values obtained in the present experiments shortly after cultures were set up. When used as a method for estimating populations in stored products, only comparative figures are needed, so the validity of their method is unimpaired provided the standard technique is followed without variation.

Summary.

If adults of *Cryptolestes ferrugineus* (Steph.), of which the females have already been fertilised, are cultured on flour at low densities, the rate of oviposition is independent of the density of adults and juvenile mortality is negligible. At high densities there is a heavy mortality amongst eggs and young larvae, and there is evidence that, providing no great degree of conditioning of the medium has occurred, an equilibrium is reached with the number of eggs *plus* larvae present at any time being independent of the density of adults. Cannibalism is undoubtedly a major

cause of the juvenile mortality under these conditions. Where crowding of adults is so great that conditioning of the medium becomes important, the number of eggs *plus* larvae present at a given time after the cultures have been set up decreases with increasing density of adults, there being some indication of an inverse linear relation.

Cultures of *C. ferrugineus* on a mixture at 70 per cent. R.H. of sound grain and grain already slightly damaged by *Cryptolestes* are capable of initiating dense infestations in the laboratory, at least at moderate and high temperatures. Under optimal conditions the infestations develop within 15 days. Values deduced for the pre-adult period at various temperatures were similar to those obtained by Rilett (1949). At 17°C., development was extremely slow but the low temperature did not increase mortality. Cannibalism and competition by adults and fully developed larvae appear to play an important part in limiting the production and survival of the juvenile stages.

Sound grain at 70 per cent. R.H. is unsuitable as a medium for culturing *Cryptolestes* in the laboratory but it becomes suitable after relatively small additions of flour and damaged grains. At 33°C. at least, a point is suddenly reached where an infestation is initiated, the critical feature being increased survival of larvae rather than an increase in the number of eggs laid. An increase in humidity has a similar effect to adding food.

Estimates of the production of CO₂ at various temperatures gave values higher than previously reported. From 21 to 33°C. the rate of increase of the respiration rate and the increase in the speed of development are proportional to rise in temperature. This is in accord with other indications that *C. ferrugineus* has a high optimum temperature.

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A STUDY OF THE ASSOCIATION BETWEEN MOORLAND VEGETATION
AND BREEDING SITES OF *CULICOIDES* (DIPTERA,
CERATOPOGONIDAE).

By D. S. KETTLE *

Midge Control Unit, Zoology Department, Edinburgh University.

During recent years a good deal of work has been done on the control of the man-biting midge, *Culicoides impunctatus* Goetgh., in Scotland. This species, which is normally univoltine, has only a relatively short life as a winged adult, and spends about ten months of the year as a vermiform larva living in wet moorland peat. Therefore the obvious approach to the control of this pest is to attack the larvae in their breeding sites, and considerable success has been obtained by this method (Kettle, Nash & Hopkins, 1956; Kettle & Parish, 1957; Kettle, Parish & Parish, 1959).

One difficulty in the way of the widespread use of this technique is that of recognising a breeding site. Breeding sites can be identified by sampling the soil and finding larvae, but this method is impractical as a preliminary to the application of insecticide because it takes far longer than the actual treatment. Some quicker method is required by which the breeding sites can be recognised on inspection. The only hope of providing such a solution lies in showing that breeding sites of *C. impunctatus* carry a distinctive flora. In looking for this association, particular attention was paid to perennial plants or those whose presence can be identified readily at all seasons, since control measures are most likely to be applied in autumn and early spring, when much of the vegetation is dormant.

It has been shown elsewhere (Kettle & Lawson, 1952) that *C. impunctatus* is almost entirely (99 per cent.) restricted to wet areas of bogland bearing *Sphagnum* and *Polytrichum* and is absent from marshes and swamps. A large fraction of the cost of any control scheme is that expended on insecticide and labour. Obviously then, the more precisely the breeding sites of *C. impunctatus* can be defined, the smaller the area to be treated in any scheme and the lower the cost.

In the course of work on the control of *C. impunctatus*, much data has accumulated relevant to this problem, and this is analysed in some detail in this paper. The investigations were made in three localities: Soutra Hill, Midlothian; Bannachra Muir, Dunbartonshire; and Luss, Dunbartonshire. On Soutra, observations were made on two separate sites (1 and 2). As the analysis of these has been more exhaustive, they will be considered first, after a brief review of the techniques used.

Techniques.

Soil sampling.

The technique of sampling soil, its subsequent treatment to recover *Culicoides* larvae, and their identification, have already been dealt with in the earlier publications quoted above.

Topographical and botanical survey.

On Soutra Site 1 a tacheometric survey was carried out using a Watts Quickset level. Readings were taken at 10-yd. intervals along traverses 10 yd. apart.

* Now at the Royal College, Nairobi, Kenya.

Additional readings were taken where there was any sudden change in height, as at the edge of the raised bog. For purposes of plotting, it was assumed that the ground sloped evenly between adjacent readings. By good fortune a bench mark (1,080.1 ft.) was available within 100 yd. of the east edge of the site so that it was possible to relate levels to mean sea-level.

For the botanical survey, the ground was divided into squares (10 yd. \times 10 yd.) and the vegetation mapped. It was not intended to make a comprehensive botanical survey; what was required was a map giving the distribution of the larger, more obvious and more abundant members of the plant community. A selection of the more characteristic plants was collected and identified. These identifications were subsequently corrected and confirmed either by Dr. A. Melderis of the

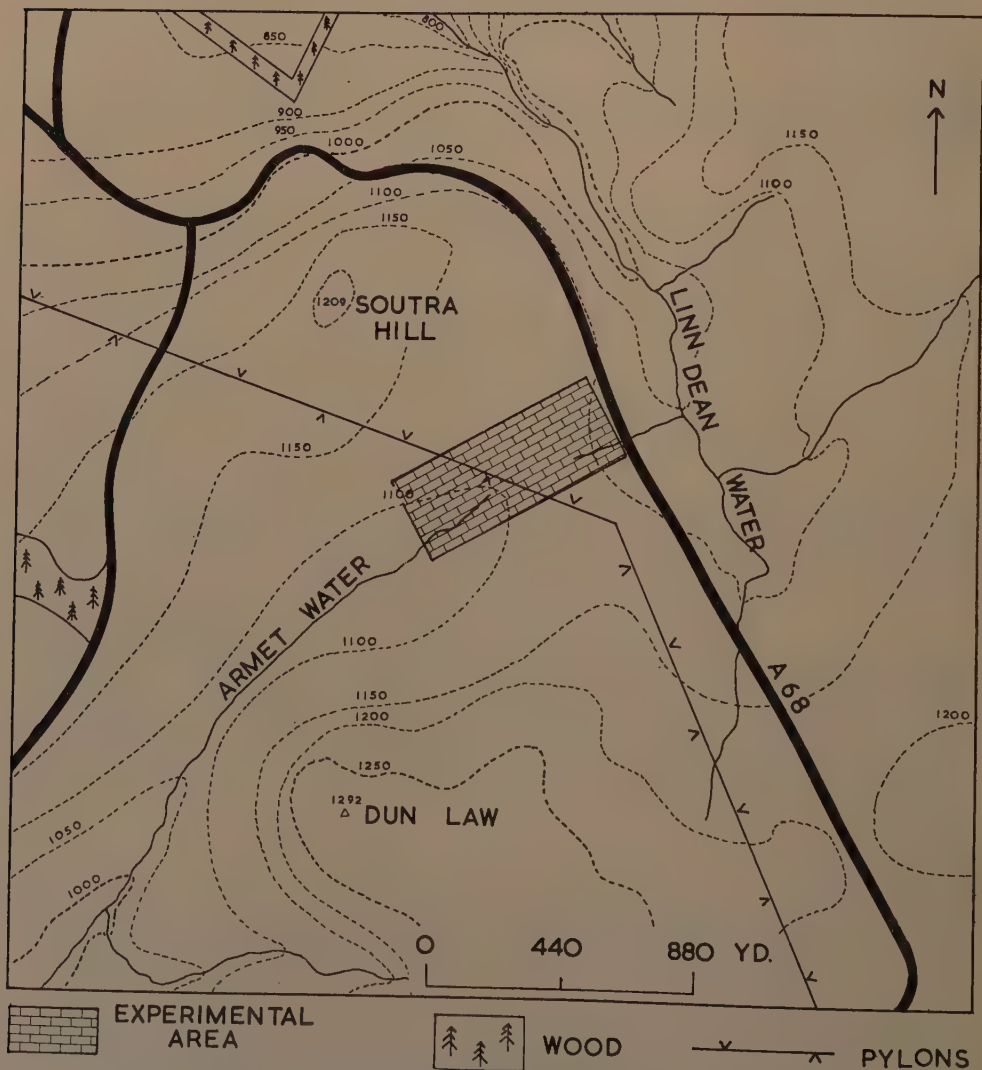


Fig. 1.—Location and general environs of the experimental area on Soutra Hill.
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Botany Department, British Museum (Natural History) or Mr. D. M. Henderson of the Royal Botanic Gardens, Edinburgh. I am extremely grateful to them for their co-operation in this way.

Soutra: General description.

The Soutra sites (fig. 1) are situated in the south-east corner of Midlothian, at its junction with Berwickshire and East Lothian, on a high-level saddle (1,100 ft. above m.s.l.) between Soutra Hill (1,209 ft.) to the north and Dun Law (1,292 ft.) to the south. Eastwards, the saddle slopes towards the valley of the Linn Dean Water, to which it supplies a tributary, around the source of which site 1 is located. Westwards, the land drains into the Armet Water, with the source of which site 2 is placed in close association.

The south-eastern slope of Soutra Hill is mainly acid grassland, with rushes in the wetter parts. On the lower, north-eastern slopes of Dun Law, where it joins the saddle, there has developed a small, raised bog about 30 acres in area. This extends across the saddle to form the southern boundary of both sites 1 and 2, but does not extend further east or west of the experimental sites.

Soutra: Site 1.

Vegetation.

There are three main zones of vegetation on site 1. To the south, in association with the raised bog, bogland plants are dominant (zone I); to the north is acid grassland (zone III), and between the two, constituting what is therefore termed the intermediate zone, is an area characterised by vegetation that prefers a much wetter habitat than either of the foregoing (zone II). (See fig. 2.)

The bogland zone (I) breaks down into three parts:

(1) Raised bog, with five main components, namely, *Calluna vulgaris*, *Eriophorum vaginatum*, *Trichophorum caespitosum*, *Cladonia sylvatica* and *Sphagnum* spp. This has a relatively smooth, gently sloping surface and ends abruptly in a low cliff of bare peat. It is the most clearly defined area of all.

(2) A belt of vegetation with a very irregular surface, which resembles a much-dissected raised bog from which the lichen (*C. sylvatica*) and deer sedge (*T. caespitosum*) have disappeared and been replaced by *Deschampsia flexuosa* and *Vaccinium myrtillus*. The dominance of *Calluna vulgaris*, *E. vaginatum* and *Sphagnum* continues.

(3) An area of dense clumps of *E. vaginatum* with *Sphagnum* filling the spaces between clumps and *E. angustifolium* occupying the wetter parts. *V. myrtillus* and *D. flexuosa* occur here as well but there is no *Calluna*.

The intermediate zone (II) comprises a mixture of bogland species and moisture-loving grassland species. Among those that attain local abundance are *Carex rostrata*, *C. nigra*, *C. panicea* and *E. angustifolium*. There is one small patch of pure *Narthecium ossifragum*, and *C. echinata* occurs but never achieves local dominance. *D. flexuosa* is the only grass. *Sphagnum* forms a dense carpet throughout the zone, and *Polytrichum* is locally abundant.

The grassland zone (III) is dominated by *Anthoxanthum odoratum*, *Festuca ovina tenuifolia* and *Agrostis canina*. In damper pockets there are local concentrations of *D. caespitosa* and *Holcus lanatus*. The drainage channels usually support a dense growth of *Juncus effusus*, while the wet areas of the grassland carry local concentrations of *C. nigra* and *C. curta*. In the north-east corner there is an extensive bed of *J. articulatus*. As one moves northwards into the grassland, scattered marshland plants are encountered, such as *Luzula multiflora*, *Cardamine pratensis*, *Potentilla erecta*, *Cirsium palustre* and *Taraxacum paludosum* agg., and mosses other than *Sphagnum* and *Polytrichum* begin to dominate. *Sphagnum* becomes steadily scarcer as one continues into the grassland.

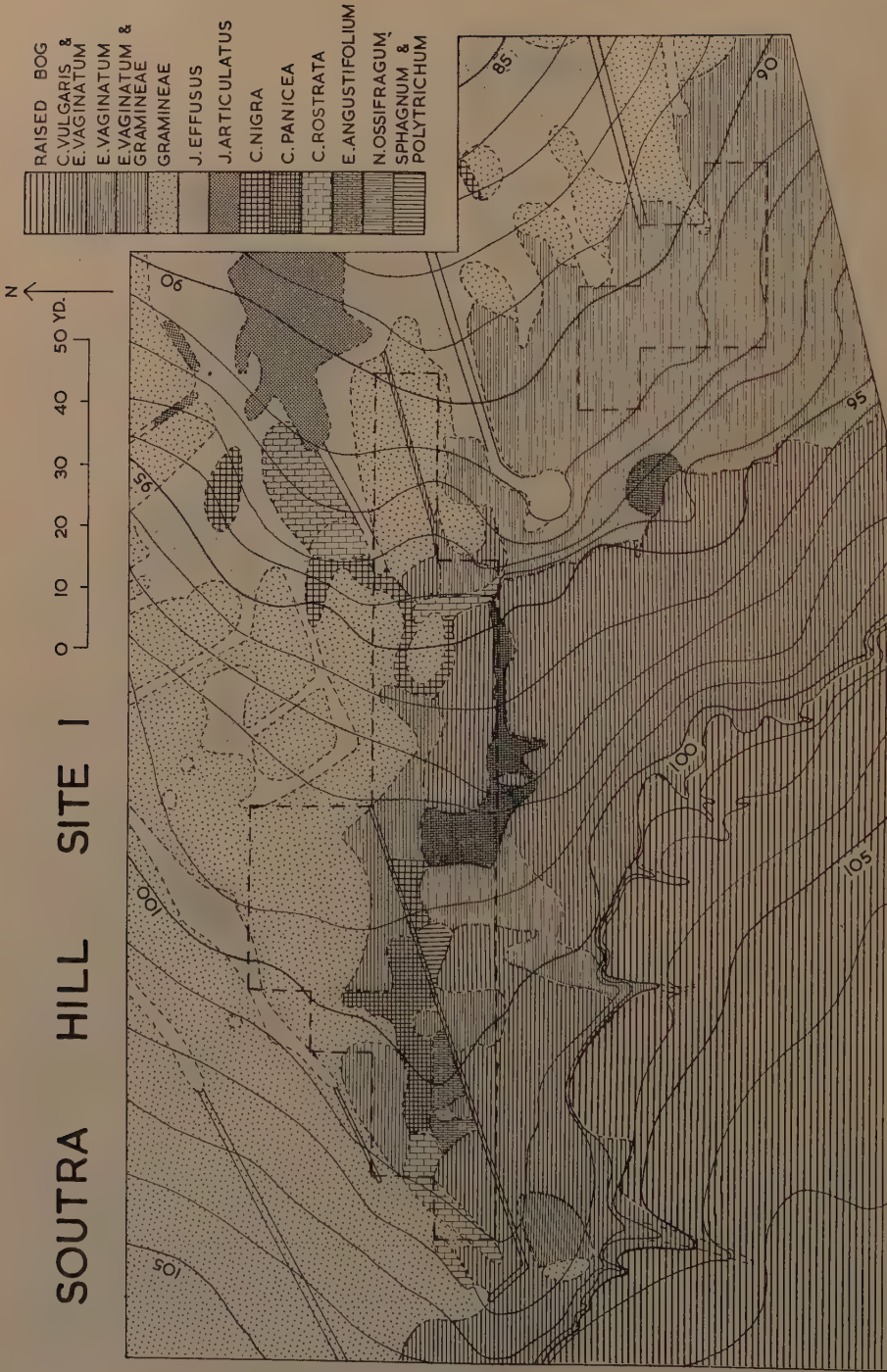


Fig. 2.—Detailed map of vegetation and contours of Soutra, site 1. Straight broken lines in centre and bottom right delimit main sampling area. Altitudes are given as feet above 1,000 ft. Bog-land (zone I) shown by horizontal lines, grassland (zone III) by dots and wet intermediate zone (zone II) by rectangles or vertical lines.

Analysis of data.

(a) *Basic data.*—The basic data on which this analysis is based were obtained from the pre-treatment sampling of the site (Kettle, Nash & Hopkins, 1956). The area sampled spanned the junction between bogland and acid grassland but did not include the raised bog. Thirty-six squares (each 10 yd. × 10 yd.) were marked out and three soil samples were removed from each square on three separate occasions between 3rd November and 3rd December 1953, thus giving a total of nine samples per square. To reduce the time that samples were retained in the laboratory before examination, only half of the squares were sampled at one time. The squares had been numbered consecutively 1–36 when set out, and squares 1–18 were assigned arbitrarily to what was termed section A and squares 19–36 to section B, the sections being sampled alternately. Squares 11 and 15 yielded very few larvae and two more squares (37 and 38) were added; in order to complete their sampling at the same time as the rest, six samples were taken from each of these squares on 3rd December. In addition, three extra samples were taken from squares 11 and 15 on 23rd November; these extra samples have been omitted when making comparisons between different squares (342 samples in Tables II and III) but included when considering other aspects of the larval distribution (348 samples in Tables I and IV). The details of the samplings were:

Date	Squares sampled	Section	Samples	No. of samples
3.xi.53	1–18	A	i–iii	52 (2 samples lost)
10.xi.53	19–36	B	i–iii	54
16.xi.53	1–18	A	iv–vi	56 (includes two replacements)
23.xi.53	19–36	B	iv–vi	54
	37 & 38		i–iii	6 (new squares)
	11 & 15		vii–ix	6 (additional samples)
27.xi.53	1–18	A	vii–ix	54
3.xii.53	19–36	B	vii–ix	54
	37 & 38		iv–ix	12
				348

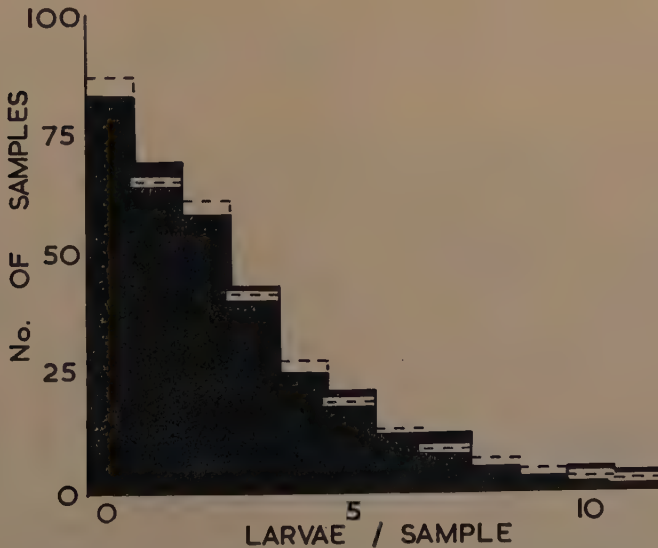


Fig. 3.—Frequency distribution of larvae of *C. impunctatus* in soil samples from Soutra, site 1. Broken lines represent the numbers expected were values of $\log_{10}(n+1)$, where n =larval numbers, normally distributed. Data from Table I.

(b) *Method of analysis*.—The frequencies with which successive values of the larval density (n) occurred are given in Table I (column 5) and fig. 3. It is clear that these are not normally distributed, but when they are transformed logarithmically, that is, to $\log_{10}(n+1)$, their distribution approximates closely to the normal (Table I). All analyses have accordingly been performed on logarithmically transformed data.

TABLE I.

Frequency distribution of larvae of *C. impunctatus* in soil samples on Soutra (site 1).

No. of larvae per sample (n)	Range of n in terms of standard deviation (s)	Per cent. of population	Expected frequency (f')	Observed frequency (f)	$f'-f$
0	< -0.679	25.0	87.0	83	+ 4.0
1	-0.679 to -0.149	18.8	65.4	69	- 3.6
2	-0.149 to $+0.288$	17.5	60.9	58	+ 2.9
3	$+0.288$ to $+0.619$	11.9	41.4	43	- 1.6
4	$+0.619$ to $+0.813$	7.9	27.5	25	+ 2.5
5	$+0.813$ to $+1.104$	5.4	18.8	21	- 2.2
6	$+1.104$ to $+1.292$	3.7	12.9	12	+ 0.9
7	$+1.292$ to $+1.456$	2.5	8.7	12	- 3.3
8	$+1.456$ to $+1.605$	1.9	6.6	5	+ 1.6
9	$+1.605$ to $+1.735$	1.3	4.5	3	- 2.0
10	$+1.735$ to $+1.856$	0.9	3.1	5	
11	$+1.856$ to $+1.965$	0.7	2.4	4	
12+	$> +1.965$	2.5	8.7	8	+ 0.7
Total		100.0	347.9	348	

The expected frequencies are calculated from the normal distribution characterised by a mean of 0.449 and a standard deviation of 0.330, these being the values derived from the data after transformation to $\log_{10}(n+1)$.

Test of agreement between expected and observed frequencies: $\chi^2=3.225$, d.f.=10, $P=0.95$ to 0.98.

(c) *Results*.—The first analysis (Table II) tested the homogeneity of the larval distributions both in space (between squares) and in time (between sampling occasions—same squares). Not unexpectedly there were highly significant differ-

TABLE II.

First analysis of variance, Soutra (site 1).

Source of variance	Degrees of freedom	Sum of squares	Mean square	F
Between sampling squares	37	8.413482	0.227391	2.519***
Between groups ..	4	2.820647	0.705162	7.811***
Within groups ..	33	5.592835	0.169480	1.877**
Between sampling occasions (same sampling squares)†	4	1.967390	0.491847	5.448***
Residue	300	27.084133	0.090280	
Total ..	341	37.465005		

In all Tables, the signs *, ** and *** indicate probabilities (P) < 0.05, 0.01 and 0.001, respectively.

† For explanation of allotment of degrees of freedom, see p. 393.

ences between squares but rather surprisingly there were similar differences between sampling occasions. This will be discussed later, while for the present attention will be concentrated on the spatial difference.

Presumably this difference between squares reflects the response of *C. impunctatus* to varied soil conditions, which themselves might be expected to influence the nature of the vegetation, although there is no reason why the plant- and insect-distribution should coincide. To test this, the squares were grouped according to the nature of their dominant vegetation and the analysis repeated. The groupings are detailed in Table III; the breakdown of the variance between squares into two portions, between vegetation groups and within such groups, is shown (indented) in Table II. The result was highly encouraging. The variance between groups

TABLE III.

Groupings of sampling squares according to dominant vegetation, Soutra (site 1).

Group	Dominant vegetation	No. of squares	No. of samples	Total larvae	Arithmetic mean (\bar{n})	Mean $\log_{10} (n+1)$
1	<i>C. vulgaris</i> & <i>E. vaginatum</i>	6	54	124	2.30	0.381
2	<i>E. vaginatum</i> (tussocks) ; no <i>C. vulgaris</i>	7	63	184	2.92	0.482
3	<i>E. vaginatum</i> & <i>E. angustifolium</i> ..	7	63	125	1.98	0.375
4	No <i>Eriophorum</i> , eastern strip ..	7	63	307	4.87	0.625
5	No <i>Eriophorum</i> , remainder	11	99	212	2.14	0.402
	All groups	38	342	952	2.78	0.449

n =number of larvae per sample.

was highly significant, much exceeding that between squares belonging to the same group (within-group variance), which was, however, significant. An association between the nature of the plant cover, and larval density, is thus suggested.

For more detailed analysis it is necessary to forget the existence of the squares and to allocate each sample to a particular plant association, because the boundaries of the squares do not coincide with those of the plant communities (fig. 2). Fortunately a precise record had been kept of the location of each sample within the squares so that this allocation could be readily achieved.

For this purpose, 19 different plant associations were recognised (Table IV), which could be allotted to the three main vegetational zones delimited earlier. When the data for larval populations in these were analysed it was found that only the difference between zones was highly significant while the variation between groups of the same zone was within the limits of experimental error (Table V). This means that the distribution of larvae of *C. impunctatus* can be correlated with that of the surface vegetation.

The first conclusion to be drawn is that larvae of *C. impunctatus* avoid the wet intermediate zone (II) and are more abundant in the bogland and acid grassland on either side (Table IV). Secondly, on this evidence there is no suggestion of selection within zones. *C. impunctatus* appears to differentiate between zones but to be insensitive to changes within the zones, as shown by variations in flora.

Lastly, and somewhat unexpectedly, larvae are significantly more numerous ($P < 0.02$) in acid grassland (zone III) than in bogland (zone I), in spite of the fact that *C. impunctatus* is usually associated with bogland. This result needs some qualification. The belt of acid grassland sampled is in some respects transitional between grassland and bogland, in that it contains many patches of *Sphagnum* and *Polytrichum*, to which the larval sampling was largely restricted (150 samples out of 162—see Table VI, zone III). Nevertheless, the larvae were not confined to these mosses because they were found just as abundantly (3.17 larvae per sample) in the 12 samples from which the mosses were absent.

TABLE IV.

Grouping used in second analysis of variance of numbers of larvae of *C. impunctatus*, Soutra (site 1).

Zone	Group	No. of samples	Total larvae	Arithmetic mean (\bar{n})	Mean $\log_{10} (n+1)$
I (Bogland)	A <i>C. vulgaris</i> ++, <i>E. vaginatum</i> ±	7	12	1.71	0.351
	B " ++, " ++	15	25	1.67	0.344
	C " +, " ++	11	27	2.45	0.388
	D " +, " +	19	39	2.05	0.379
	E " ±, " +++	7	7	1.00	0.240
	F " —, " +++	12	50	4.17	0.578
	G " —, " +++	63	184	2.92	0.482
	Zone I (all groups)	134	344	2.57	0.433
II (Intermediate)	H <i>E. vaginatum</i> +, <i>E. angustifolium</i> +	13	23	1.77	0.324
	I <i>E. angustifolium</i>	5	3	0.60	0.181
	J " , <i>C. panicea</i> , <i>C. nigra</i>	4	7	1.75	0.389
	K <i>C. panicea</i> , <i>Erica tetralix</i>	16	25	1.56	0.291
	L " +++ , <i>E. vaginatum</i> ±, grass	2	7	3.50	0.650
	M <i>N. ossifragum</i>	3	4	1.33	0.301
	N <i>C. rostrata</i>	9	9	1.00	0.217
	Zone II (all groups)	52	78	1.50	0.298
III (Grassland)	O Gramineae,	78	221	2.83	0.464
	P " , <i>E. vaginatum</i> ±	65	248	3.82	0.552
	Q " , " +	5	13	2.60	0.336
	R " , <i>Sphagnum</i> , <i>Polytrichum</i>	6	20	3.33	0.604
	S <i>C. nigra</i>	8	40	5.00	0.657
	Zone III (all groups)	162	542	3.35	0.510
	All zones	348	964	2.77	0.449

Increasing relative abundance of plants shown by signs (—, ±, +, ++ and +++).
 n = number of larvae per sample. All individual and combined zone-means weighted.

(d) *Additional data*.—From the evidence above there is no reason to believe that breeding of *C. impunctatus* does not extend further into the wet areas of the acid grassland. There are, however, additional data available. At various times, samples were taken in the vicinity of site 1 (Table VII). On the raised bog (a,

TABLE V.

Second analysis of variance of numbers of larvae of *C. impunctatus*, Soutra (site 1).
Data of Table IV.

Source of variance	Degrees of freedom	Sum of squares	Mean square	F
Between zones	2	1.826641	0.913320	9.92***
Between groups (same zone)	16	1.985751	0.124109	1.35
Between sampling occasions	5	3.730445	0.746089	8.10**
Zone I	5	1.874938	0.374988	4.07**
Groups A-D inclusive	4	0.433101	0.108275	1.18
Groups E-G inclusive	5	1.310952	0.262190	2.85*
Zone II	5	0.404926	0.080985	0.88
Zone III	5	1.016730	0.203346	2.21
Interaction sampling occasions × zones	10	1.392790	0.139279	1.51
Residue	314	28.907614	0.092062	
Total	347	37.843241		

For symbols see Table II.

Soutra (site 1) was sampled six times (sections A and B three times each). Each zone was sampled on each occasion, giving 6 samplings and 5 degrees of freedom (d.f.). No samples were taken from groups A-D on one occasion, so that these were sampled only five times and have therefore 4 d.f.

in Table VII), larvae were present at low density, less than one per sample. In the other *Sphagnum*-dominated areas (b-g), *C. impunctatus* was more abundant, the 79 samples yielding 463 larvae (5.9 per sample). But in the *Juncus*-dominated areas (h-j), the larval density was again low, and 27 samples produced only 36 larvae (1.3 per sample). It would appear, therefore, that breeding by *C.*

TABLE VI.

Density of larvae of *C. impunctatus* per sample in different vegetation zones in relation to type of moss present, Soutra (site 1).

Zone	<i>Sphagnum</i> only	<i>Polytrichum</i> only	<i>Sphagnum</i> and <i>Polytrichum</i>	Neither moss
I. Bogland ..	2.60 (95)	1.50 (4)	2.60 (35)	— (0)
II. Intermediate	1.44 (27)	3.67 (3)	1.05 (20)	2.00 (2)
III. Grassland	3.24 (99)	5.17 (6)	3.33 (45)	3.17 (12)
Total ..	2.76 (221)	3.92 (13)	2.62 (100)	3.00 (14)

Number of samples given in brackets.

impunctatus occurs most abundantly at the edge of the raised bog and extends in smaller numbers on to it and into the acid grassland. It is perhaps unfortunate that sampling of the latter was confined to the neighbourhood of the raised bog. Samples taken from other wet grassy areas devoid of *Sphagnum* have produced only occasional *C. impunctatus* larvae (Kettle & Lawson, 1952).

TABLE VII.
Additional observations on vegetation and larvae of *Culicoides* and related genera in the vicinity of site 1 on Soutra.

Reference letter	Location	Date of observation	Vegetation	Zone	No. of samples examined	<i>Culicoides impunctatus</i> Goetgh.	<i>Ischlea</i> spp.	<i>Ceratopogon</i> spp.	<i>Culicoides albicans</i> (Winn.)	<i>C. heliophilus</i> Edw.	<i>C. obsolatus</i> (Mg.)	<i>C. cubitalis</i> Edw.	<i>C. pulicaris</i> (L.) group	<i>C. truncorum</i> Edw.	<i>C. pallidicornis</i> (Kieff.)
a	Raised bog	18.v.54	See text	I	18	16	7	—	—	—	—	—	—	—	—
b	W. of site 1	28.ix.53	<i>Sphagnum</i> , <i>Polytrichum</i> , <i>Narthecium</i>	II	3	24	20	1	10	—	—	—	—	—	—
c	SE. of site 1	14.x.53	<i>Sphagnum</i>	I	3	8	4	1	—	—	—	—	—	—	—
d	Near c	28.ix.53	<i>Sphagnum</i> , other moss	I	2	22	17	2	—	—	—	—	—	—	—
e	E. of site 1	8-26.x.53	<i>Sphagnum</i> , <i>E. vaginatum</i>	I	47	277	31	7	3	—	—	—	—	—	—
f	SE. of site 1	22.x.53	<i>Sphagnum</i> , <i>Polytrichum</i> (clearing in 'i')	III	6	32	4	—	—	—	—	—	—	—	—
g	E. of site 1	8.xii.54	<i>Sphagnum</i> , <i>J. effusus</i>	III	18	100	4	2	—	3	9	24	1	—	—
h	N. of site 1	6.i.54	<i>J. effusus</i>	III	6	23	11	—	5	12	2	48	12	—	—
i	SE. of site 1	22.ix.53	<i>J. articulatus</i>	III	3	2	—	—	—	2	2	—	—	—	—
j	E. of site 1	8.xii.54	<i>J. articulatus</i>	III	18	11	3	2	—	29	8	117	16	3	3

TABLE VIII.

Number of larvae of *Culicoides* and related genera per 100 samples in each zone, and, in brackets, actual number, from which χ^2 was calculated, Soutra (site 1).

Zone	<i>C. impunctatus</i>	<i>C. albicans</i>	<i>C. heliophilus</i>	<i>C. cubitalis</i>	<i>C. obsoletus</i>	<i>Ceratopogon</i> spp.	<i>Ischelea</i> spp.	No. of samples
I	257 (344)	7 (10)	1 (2)	0 (0)	0 (0)	16 (22)	97 (130)	134
II	150 (78)	29 (15)	4 (2)	6 (3)	0 (0)	10 (5)	138 (72)	52
III	335 (542)	3 (5)	11 (18)	4 (6)	10 (16)	9 (15)	94 (153)	162
Total	(964)	(30)	(22)	(9)	(16)	(42)	(355)	348
χ^2	51.60***	30.51***	11.45**	6.49	18.59***	4.42	8.18*	

The transition from bogland to grassland involves two major changes, environmental and biotic. First, there is a change in the constitution of the soil, and secondly, there is the possibility of increasing competition from other species of *Culicoides*. It can be seen from Table VII that *C. impunctatus* is almost the only species of *Culicoides* to occur in bogland (a, c-e) while in the wetter intermediate zone II it is found with *C. albicans* (b), whereas in acid grassland several other species occur and may be more abundant (h-j), except where the bogland flora persists in small pockets (f) and there *C. impunctatus* occurs alone. One set of samples (g) seem to have been taken from an area of transition between bogland and grassland.

Distribution of other species of Culicoides and related genera.

The distributions of some other Ceratopogonids in the three zones are given in Table VIII. Values of χ^2 have been calculated to test whether the densities are similar in the three zones. *C. albicans* (Winn.) shows marked preference for the wet intermediate zone (II) (see also Table VII, b); this preference is also shown, to a lesser degree, by *Isohelea* spp., although here the possibility of more than one species' being included in the data may mask any preference. *C. obsoletus* (Mg.) and *C. heliophilus* both prefer the acid grassland (zone III). The data on *C. cubitalis* and *Ceratopogon* spp. from site I are inadequate to reveal a preference, but from Table VII it is clear that *Culicoides cubitalis* prefers zone III.

Short-term fluctuations in larval density.

In the first analysis of variance (Table II), it was found that there were highly significant differences between the larval populations in the same squares from one sampling to the next. This result was unexpected because these samplings were made in the late autumn, at the end of adult activity, and it was expected that the larval population would remain steady or decrease slowly due to deaths. Kettle, Parish & Parish (1959) showed that these changes were not due to insecticidal repellency (and the present analysis is restricted to pretreatment data), to delayed hatching of eggs, or to changes in technique, and concluded that they were

TABLE IX.

Mean values of numbers of larvae of *C. impunctatus* per sample (logarithmically transformed) in different collections from Soutra (site 1), given as differences from zone mean (penultimate right-hand column), multiplied by 1,000. Number of samples given in brackets. For method of adjustment, see p. 393.

Zone	Nov. 3	Nov. 10	Nov. 16	Nov. 23	Nov. 27	Dec. 3	Zone mean	Mean deviation (ignoring sign)
I	+22 (21)	+249 (15)	-156 (21)	-101 (24)	-27 (24)	+75 (29)	0.433 (134)	105
II	-23 (14)	+3 (1)	-82 (20)	+91 (4)	+107 (12)	+304 (1)	0.298 (52)	102
III	-44 (18)	+68 (38)	-67 (14)	-55 (38)	-127 (18)	+97 (36)	0.510 (162)	76
All (unadjusted)	-38 (53)	+153 (54)	-152 (55)	-42 (66)	-51 (54)	+115 (66)	0.449 (348)	92
All (adjusted)	-12	+117	-106	-63	-31	+91		70

due to a response by the larvae to some widespread oscillating factor, possibly soil-water level. The previous analysis had as its basic unit the individual sampling square but it has already been shown above that the true unit is the vegetational zone. It is necessary, therefore, to repeat the analysis, incorporating this information.

The sampling squares were allotted to one of two sections (A and B), which were sampled alternately. Each section was sampled three times, giving two degrees of freedom between samplings in each section and four in all (Table II). When the analysis is based on the three zones there are five degrees of freedom because each zone was sampled on each of the six occasions (Table V).

The data are set out in Table IX. In the unadjusted figures (penultimate row) the actual mean larval density has been compared directly with the over-all mean. This ignores the fact that a different number of samples was taken from each zone on each sampling occasion, and the figures must be adjusted to eliminate the zonal influence, before the different occasions can be accurately compared. This has been made by multiplying the number of samples in each zone by the mean larval density to obtain the expected larval catch, summing the three products and dividing by the total number of samples to get the expected mean catch. This value can be compared with that observed. Although more accurate, this adjustment does not materially affect the result, as may be seen by comparing the adjusted and unadjusted values in Table IX.

The data are plotted in fig. 4. It will be noted that the larval densities in all zones fluctuate in unison. A possible explanation is that sampling was not

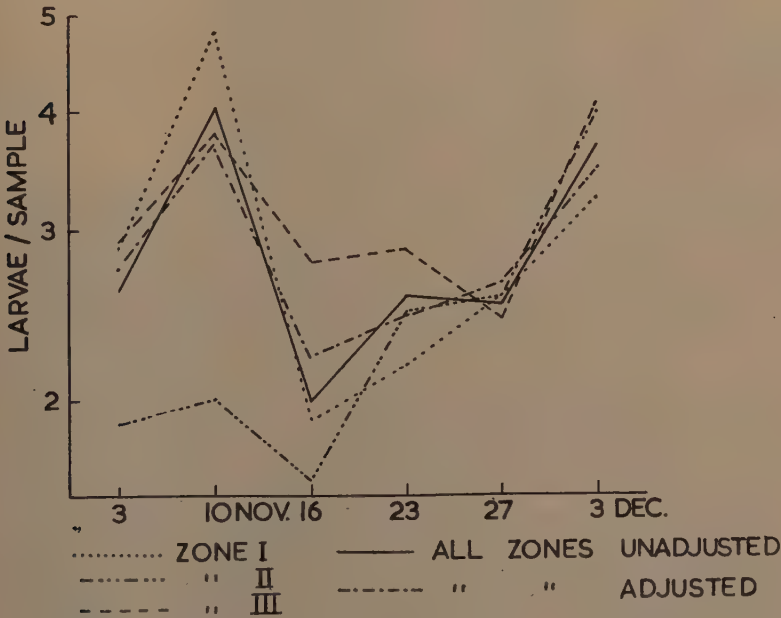


Fig. 4.—Fluctuations in numbers of larvae of *C. impunctatus* on successive dates in each vegetation zone; Soutra, site 1. Data from Table IX.

completely at random but was randomised over apparently suitable terrain; if, in response to some factor, larvae moved into 'unsuitable' ground, there would be an apparent decrease in larval density and *vice versa*. The responsible factor might

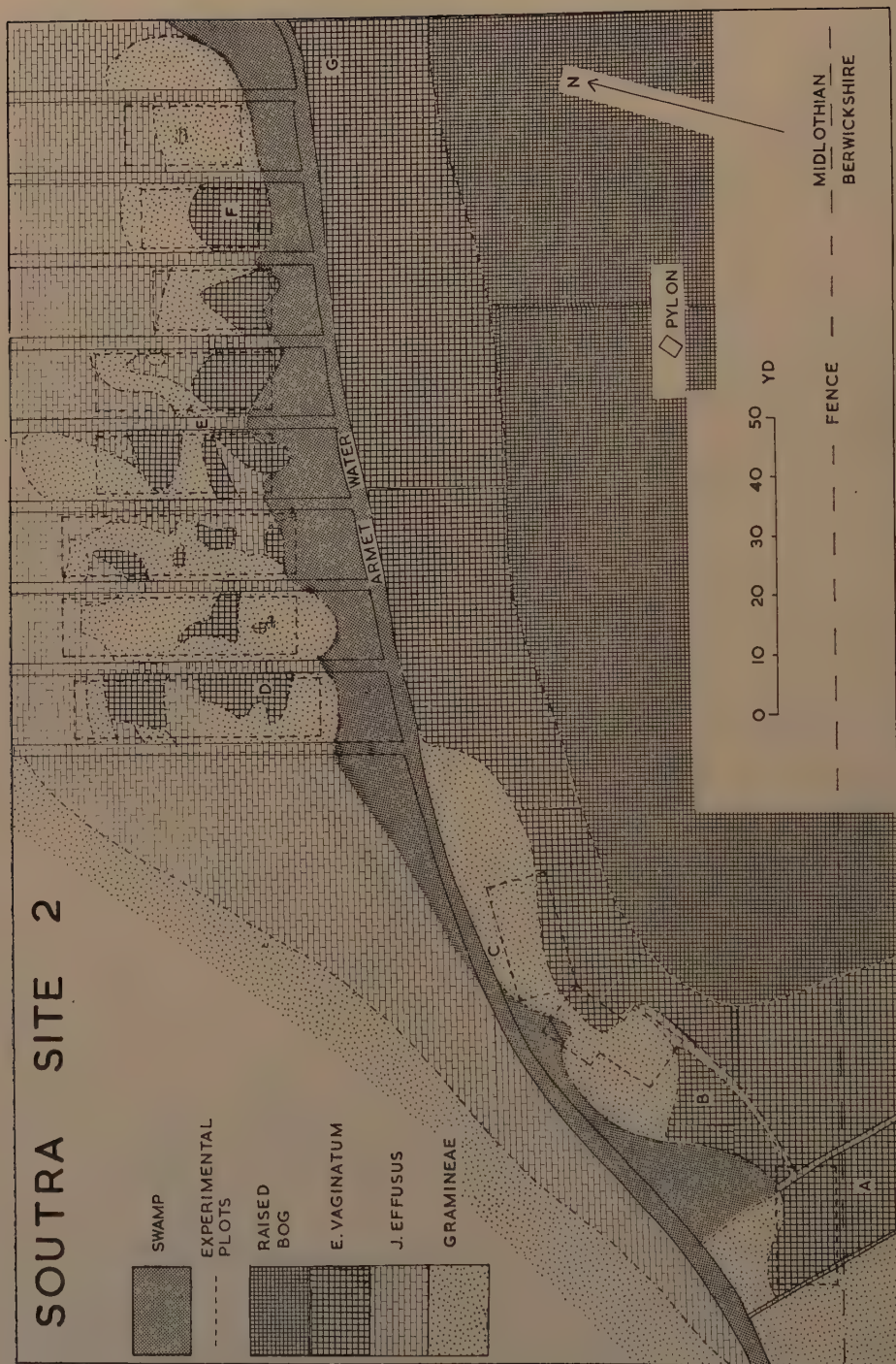


Fig. 5.—Map of main vegetational zones on Soutra, site 2. Straight, broken line delimits sampling squares, of which 28 and 29 are at bottom left.

be soil-water level: as this rose, larvae would tend to move to areas 'normally' too dry and when it fell they would move into areas previously too wet. The avoidance of zone II by larvae of *C. impunctatus* is a sign that they do not like very wet soil.

On uneven moorland, a small lateral movement would enable larvae to keep their positions relative to the soil-water level and the soil surface. If that were so, the fluctuations should be more pronounced in the zone with the most broken ground. This is indeed so. In Table V the variance between sampling occasions is given for each zone and only that for zone I is highly significant. This zone is strewn with clumps of *Eriophorum vaginatum* and *Calluna vulgaris*, and when the groups of samples taken in it are divided into two parts, one (E-G) being the more and the other (A-D) the less tussocky, only the former shows significant variability. In this narrowing-down process the degrees of freedom have remained unaltered (5 and 314) yet the *F* ratios have steadily decreased (8.10, 4.07 and 2.85). This means that at every subdivision a useful contribution to the variance has been lost. That is, the difference between sampling occasions occurs in all zones (as fig. 4 graphically illustrates) but it is more pronounced where the ground is more broken.

Soutra: Site 2.

Vegetation and position of sample plots.

The same three zones as were found on site 1 can be recognised on site 2. Zone I occupies the southern half of the site and once again it can be subdivided into three parts:

(1) The raised bog occupies the south and south-eastern corner, and carries the same flora as on site 1.

(2) To the north, between the raised bog and the Armet Water, there is a belt of bogland which corresponds to part two of zone I on site 1 and has a very similar flora. Two of the sampling squares (28 and 29) were located in this region (south-west corner), which also penetrates into three other squares.

(3) An extension of zone I across the Armet Water, where it forms scattered patches of bogland between the Water and the extensive acid grassland. This corresponds approximately to part (3) of zone I on site 1 (p. 383), and occurs in many of the sampling squares. In later analysis reference will be made to these two parts of zone I, one fringing the raised bog and the other adjacent to zone III.

On this site, zone II is very much wetter than on site 1. It extends in a swampy belt on either side of, and including, the Armet Water. It carries a luxuriant growth of sedges, rushes and swamp grasses, and forms the southern edge of many squares.

The greater part of this site was acid grassland (zone III). It was traversed from north to south by many parallel drainage channels, about two yd. wide and two to three ft. deep, occurring at intervals of approximately 14 yd. Consequently, most of the sampling squares were arranged in short columns placed between adjacent ditches. Zone III was readily divided into two parts, one of which was dominated by *J. effusus* and the other by Gramineae.

In later analysis, reference will be made to the sampling squares occurring in three areas: (i) the two squares in the south-west corner; (ii) the four squares about 20 yd. away to the north-east; and (iii) the greater number of the squares located north of the Armet Water.

Analysis of data.

In all, 30 squares (10 yd. \times 10 yd.) were marked out but, later, two pairs of adjacent squares were amalgamated, forming two sampling rectangles (20 yd. \times 10 yd.), thus giving, in all, 28 effective sampling units. It was intended to remove three samples from each square on three separate occasions but for various reasons

there were deviations from this simple arrangement and the actual samplings were as shown below.

Date	Squares sampled	Samples	No. of samples
10.iii.54	1-24	i-iii	72
17.iii.54	1-20 (excl. 3 & 4)	iv-vi	54
	25-29	i-vi	30
23.iii.54	1-24*	vii-ix	63
	25-29	vii-ix	15
	30	i-ix	9
2.iv.54	21 & 22		9
Total			252

* 3 pairs of squares were amalgamated (3+4, 21+22, 23+24).
Later 21 and 22 were separated.

It was first necessary to decide whether to transform the data. Logarithmic transformation did not produce a normal frequency distribution (fig. 6), possibly because the observations were made up of several normal distributions with different means and/or variances. The data were therefore allocated to one of the following three groups, according to the zone from which they were collected: *E. vaginatum* + *Carex rostrata*; *J. effusus*; and Gramineae + *C. echinata*. The frequencies were



Fig. 6.—Frequency distribution of larvae of *C. impunctatus* on Soutra (site 2). The broken lines indicate the numbers expected were the logarithmic transformations of larval numbers normally distributed.

normally distributed in the first two groups but not in the third, in which there was an excess of samples containing no larvae. When these zero values were omitted, the remainder were normally distributed (fig. 7).

The data were transformed logarithmically and analyses carried out on values of $\log(n+1)$, where n is the number of larvae per sample. The first analysis (Table X, A) was based on the original data from the sampling squares. The

variation between squares was highly significant, but not that between the four sampling occasions, and therefore in subsequent analyses the variance between occasions was not calculated separately but was left in the residual variance.

The second analysis (Table X, B) showed that although site 2 was composed of three distinct areas, the difference between them was insignificant, and could not, therefore, be the source of the variance between plots.

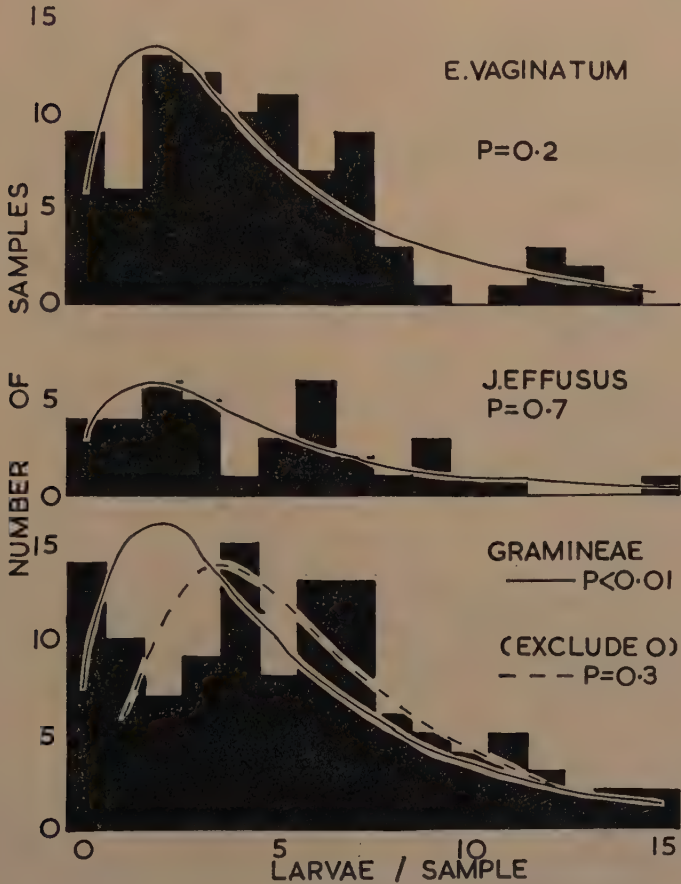


Fig. 7.—Frequency of distribution of larvae of *C. impunctatus* in various vegetational groups of Soutra, site 2. Continuous line indicates expected distribution were the logarithmic transformations of larval numbers normally distributed. Broken line in bottom histogram refers to similar distribution but omitting samples with no larvae. P measures the significance of the difference between expected and observed values.

On site 1, the differences between squares had been associated with various plant associations. On site 2, eight vegetation groups were recognised (Table XI) and the data re-analysed, but without demonstrating significant differences between groups (Table X, C).

On site 1, the larval density of *C. impunctatus* was correlated with broad vegetational divisions. The data were therefore reorganised as shown in Table XII. The source of the samples could be classified in two ways, using either the flowering plants or the mosses as the criterion. *Sphagnum* and *Polytrichum* abounded

TABLE X.

Analyses of variance of data for numbers of larvae of *C. impunctatus*, Soutra (site 2).

Analysis	Source of variance	Degrees of freedom	Sum of squares	Mean square	F
A	Between squares	27	6.827335	0.252864	2.57***
	Between sampling occasions	3	0.475018	0.158339	1.61
	Residue	221	21.759728	0.098460	
	Total	251	29.062081		
B (See text)	Between areas	2	0.493435	0.246718	2.15
	Within areas	249	28.568646	0.114734	
C (Table XI)	Between vegetation groups	7	0.859793	0.122828	1.06
	Within groups	244	28.202288	0.115583	
D (Table XII and text)	Between moss groups	2	0.051391	0.025696	0.22
	Between flowering-plant groups	3	0.140305	0.046768	0.41
	<i>E. vaginatum</i> —zone 1, Pts. 2 v. 3	1	0.128871	0.128871	1.12
	Interaction (moss groups × flowering-plant groups)	6	0.882810	0.143802	1.25
	Residue	238	27.383688	0.115058	

throughout the sampled area, only one of the 252 samples containing neither of these mosses. The samples could come from one of three possible moss associations: *Sphagnum* alone, *Polytrichum* alone, or a mixture of the two. If classified according to flowering plants, all samples from zone I came from an *E. vaginatum* community, while samples from zone III came from parts dominated either by

TABLE XI.

Distribution of larvae of *C. impunctatus* in areas dominated by various flowering plants, Soutra (site 2).

Zone	Vegetation group	No. of samples	Total larvae	Arithmetic mean (\bar{n})	Mean $\log_{10} (n + 1)$
I	<i>E. vaginatum</i>	80	400	5.00	0.669
II	<i>C. echinata</i>	2	14	7.00	0.903
II	<i>C. rostrata</i> , <i>E. vaginatum</i> ..	7	49	7.00	0.851
III	Gramineae	110	635	5.77	0.710
III	<i>J. effusus</i>	35	187	5.34	0.684
III	<i>J. effusus</i> & Gramineae ..	8	29	3.63	0.587
I & III	<i>E. vaginatum</i> & Gramineae	7	20	2.86	0.448
I & III	<i>E. vaginatum</i> & <i>J. effusus</i> ..	3	16	5.33	0.752
	All zones ..	252	1350	5.36	0.688

n = number of larvae per sample.

J. effusus or by grasses. The last group (labelled 'Others' in Table XII) contained the few samples from zone II and those taken at the junction of two of the other three groups.

The values of the logarithmic means in the extreme right-hand column and bottom line of Table XII suggest that little is to be obtained from this arrangement; the seven mean values are remarkably uniform, ranging from 0.661 to 0.717. The analysis of variance (Table X, D) confirms this impression. The variances between moss groups, between flowering-plant groups and for the interaction between them,

TABLE XII.

Distribution of larvae of *C. impunctatus* in samples classified according to dominant flowering plant and associated moss, Soutra (site 2).

Dominant vegetation		<i>Sphagnum</i>	<i>Sphagnum</i> & <i>Polytrichum</i>	<i>Polytrichum</i>	Total
<i>E. vaginatum</i> (Zone I)	a	20	221	159	400
	b	5	40	35	80
	c	4.00	5.53	4.54	5.00
	d	0.680	0.718	0.610	0.669
Gramineae (Zone III)	a	102	124	409	635
	b	19	27	63	109
	c	5.37	4.59	6.49	5.83
	d	0.664	0.634	0.768	0.717
<i>J. effusus</i> (Zone III)	a	26	46	115	187
	b	3	12	20	35
	c	8.67	3.83	5.75	5.34
	d	0.815	0.613	0.701	0.681
Others (Zone II and junctions of other groups)	a	31	62	35	128
	b	4	14	9	27
	c	7.75	4.43	3.89	4.74
	d	0.842	0.664	0.576	0.661
Total	a	179	453	718	1350
	b	31	93	127	251
	c	5.77	4.87	5.65	5.38
	d	0.704	0.672	0.701	0.690

a=total larvae found; b=no. of samples examined; c= \bar{n} , and d=mean $\log_{10}(n+1)$, where n =no. of larvae per sample.

are all insignificant. For completeness, a comparison was made between the two areas of *E. vaginatum* in zone I. Part 2 was near the raised bog and south of Armet Water, whereas part 3 was between the Water and zone III. This difference was also insignificant.

We are left, therefore, with an unsolved problem. Site 2 has not yielded to the same analysis as site 1. There is a highly significant difference between sampling squares ($P<0.001$) but this cannot be explained in terms of varied soil conditions as revealed by the plant cover. Some light is shed on this problem by observations on sampling squares 28 and 29. These squares, in the south-west corner of the site (fig. 5), were kept untreated to reveal natural changes in the larval population of *C. impunctatus*. As part of the original insecticidal trials they were sampled on five occasions between 17th March and 26th May 1954 (Table XIII), and again monthly from November 1954 to January 1955. In the intervening period (June–September 1954, inclusive) samples were collected at monthly intervals outside but within one yard of the squares.

Both these squares were covered by the same association of plants (*E. vaginatum*) and yet No. 29 yielded consistently higher larval densities than did No. 28 (Table XIII, last column; fig. 8). The data have been analysed in Table XIV,

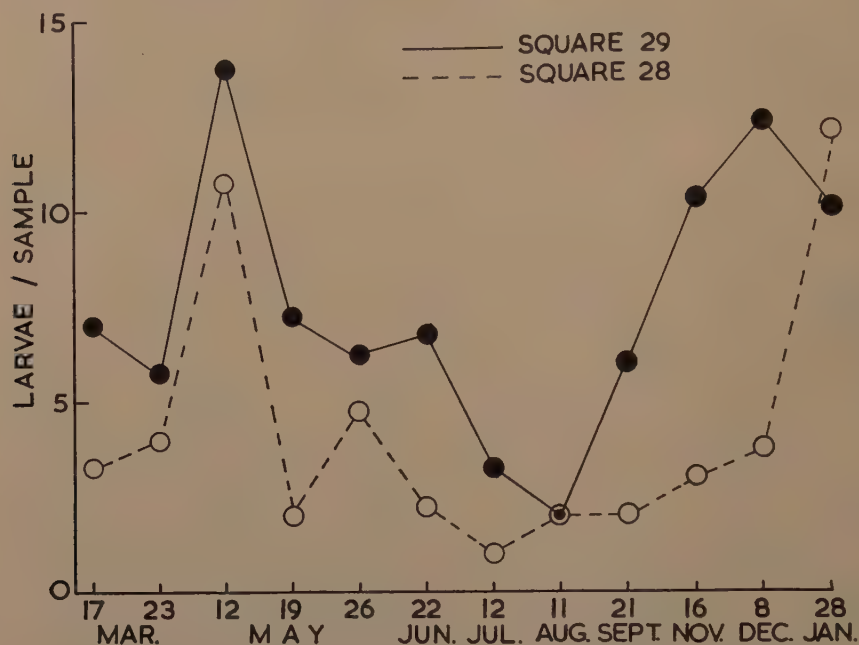


Fig. 8.—Larval densities of *C. impunctatus* in two adjacent sampling squares whose vegetation appears identical. Data from Table XIII.

TABLE XIII.

Density of larvae of *C. impunctatus* in and around squares 28 and 29, Soutra (site 2).

Square 28				Square 29			
	(a)	(b)	(c)	(d)	(e)	(f)	
Date (1954-55)	No. of samples	Total larvae	Arithmetic mean (\bar{n})	No. of samples	Total larvae	Arithmetic mean (\bar{n})	Ratio f/c
17.iii ..	6	18	3.0	6	42	7.0	2.3
23.iii ..	3	11	3.7	3	17	5.7	1.5
12.v ..	3	32	10.7	3	41	13.7	1.3
19.v ..	3	6	2.0	3	22	7.3	5.3
26.v ..	3	14	4.7	3	19	6.3	1.3
22.vi ..	3	7	2.3	3	20	6.7	2.9
12.vii ..	3	3	1.0	3	10	3.3	3.3
11.viii ..	3	6	2.0	6	12	2.0	1.0
21.ix ..	3	6	2.0	3	18	6.0	3.0
16.xi ..	3	9	3.0	3	31	10.3	3.4
8.xii ..	3	11	3.7	3	37	12.3	3.3
28.i ..	3	36	12.0	3	30	10.0	0.8
Total ..	39	159	4.1	42	299	7.1	1.73
Mean log ₁₀ ($n + 1$)			0.584				0.807

n = no. of larvae per sample.

which shows a very highly significant difference between the squares, despite their occurrence in the same plant community. It is also interesting that this difference persists throughout the period of emergence and to the end of the summer, when the new larval population appears. Clearly, although the two squares appear, to the human eye, to be reasonably similar, they are not equally attractive to ovipositing females of *C. impunctatus*.

TABLE XIV.

Analysis of variance of numbers of larvae of *C. impunctatus* found in samples in and around squares 28 and 29, Soutra (site 2).

Source	Degrees of freedom	Sum of squares	Mean square	<i>F</i>
Square 28 v. Square 29 ..	1	1·006572	1·006572	12·07***
Between sampling occasions	11	2·747865	0·249806	3·00**
Residue	68	5·669987	0·083382	
Total ..	80	9·424424		

On site 2, then, the larval distribution is affected more by differences within zones than between them. Therefore the most homogeneous units are the individual squares, and it is on this basis that differences between sampling occasions should be considered. This has already been dealt with by Kettle, Parish & Parish (1959).

Distribution of other *Ceratopogonids*.

On site 2 at Soutra, very few other species of *Culicoides* were found (Table XV), but larvae of *Isohelea* and *Ceratopogon* were present in moderate numbers. Larvae of *Isohelea* were distributed evenly throughout the four groups of flowering plants,

TABLE XV.

Distribution of larvae of other *Ceratopogonids*, Soutra (site 2).

Dominant vegetation	No. of samples	<i>Isohelea</i> spp.	<i>Cerato-</i> <i>pogon</i> spp.	<i>Culicoides</i> <i>albicans</i>	<i>C. helio-</i> <i>philus</i>	<i>C.</i> <i>cubitalis</i>
<i>E. vaginatum</i>	80	19	30	1	2	1
Gramineae	110	22	21	0	1	0
<i>J. effusus</i>	35	8	0	0	0	0
Others	27	9	3	0	0	0
Total	252	58	54	1	3	1
χ^2_P		1·71	18·87			
.. ..		Insig.	<0·001			
<i>Sphagnum</i>	31	8	4			
<i>Sphagnum</i> & <i>Polytrichum</i>	93	39	27			
<i>Polytrichum</i>	127	11	23			
Total	251	58	54			
χ^2_P		24·97	4·22			
.. ..		<0·001	Insig.			

TABLE XVI.
Additional observations on Ceratopogonid larvae in and around Soutra (site 2). For location, see fig. 5.

Collection	Vegetation	Zone	Date	Reference letter in fig. 5	No. of samples	<i>Culicoides impunctatus</i>	<i>Ischelea</i> spp.	<i>Ceratopogon</i> spp.	<i>Culicoides albicans</i>	<i>C. heliophilus</i>	<i>C. obsolitus</i>	<i>C. cubitalis</i>	<i>C. pulicaris</i> group
1 (a) (b)	<i>Sphagnum</i> , <i>E. vaginatum</i>	I	25.i.54 6 & 12.v.54	A	? 38	33 104	— 10	2 19	— —	— 4	2 —	— 22	— 2
2	<i>Sphagnum</i> , <i>E. vaginatum</i>	I	25.i.54	B	?	26	3	4	—	—	—	—	—
3	<i>Sphagnum</i> , <i>Polytrichum</i> , <i>E. vaginatum</i>	I	25.i.54	D	?	58	12	—	—	—	3	—	—
4	<i>Sphagnum</i> , <i>Polytrichum</i> , <i>E. vaginatum</i>	I	14.x.53	F	9	50	8	—	—	—	—	—	—
5	<i>Sphagnum</i> , <i>E. vaginatum</i>	I	28.ix.53	G	12	92	44	6	—	—	—	—	—
6	<i>J. articulatus</i>	III	12.v.54	C	9	1	—	—	2	2	—	21	—
7	<i>J. effusus</i> , <i>Sphagnum</i> , <i>Polytrichum</i>	III	12.v.54	E	9	3	—	—	—	1	—	1	—

thus showing a distribution similar to that of *C. impunctatus*, but those of *Ceratopogon* were unequally distributed, being more abundant in the *E. vaginatum* belt. An odd fact is that when the samples are classified according to their moss association it is the larvae of *Ceratopogon* that are evenly distributed and those of *Isohelea* that show a preference, avoiding *Polytrichum* and choosing the *Sphagnum*/*Polytrichum* association.

Other samples in and around Soutra site 2 (Table XVI) were equally deficient in other species, except for a certain amount of *C. cubitalis*. *C. impunctatus* dominated all the samples in zone I (collections 1-5), where it was associated with *Isohelea* and *Ceratopogon*. In the drainage channel (collection 7), few larvae were found. *C. cubitalis* was abundant in the absence of bogland mosses (collection 6) and also occurred in one collection (1 b) from the edge of zone I.

Bannachra, 1949.

The data available for analysis from Bannachra were collected in the early months of 1949 in connection with locating a postulated breeding site of *C. impunctatus* (Kettle, 1951). The area to be investigated was divided into 6 x 6-yd. squares and three samples removed from each square.

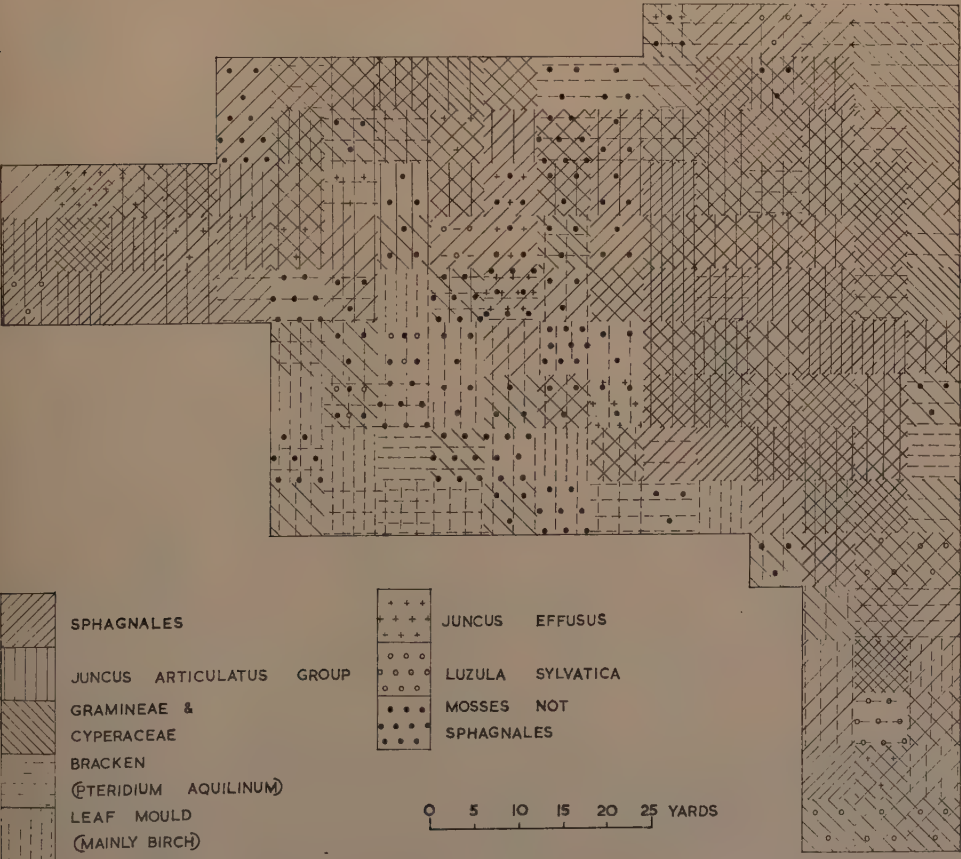


Fig. 9.—Distribution of vegetation on Bannachra site. For explanation see text.

The procedure adopted differed from that on Soutra in that sampling was not confined to apparently suitable vegetation associations. At Bannachra, samples were taken from the three major plant communities in each square. These botanical findings are plotted in fig. 9, where no attempt has been made to map the exact limits of any plant species. The symbols representing each plant sampled are spread over the whole square, and their density indicates whether the plant concerned was present in one, two or all three samples. As these investigations were carried out in the winter and early spring, no separation was made between grasses and sedges, which will be referred to as 'Gramineae.'

The area illustrated in fig. 9 was largely in woodland with the upper edge stretching into more open moorland. The ground sloped from above downwards. Water drained down the hillside near the surface until prevented by an earth-and-stone dyke which ran across the area about one-third of the way from the top in the figure. As a result, water collected on the upper side of the wall and then flowed from left to right until it found a gap in the wall and spilt down through the woodland to the bottom right-hand corner. The course of the water is indicated in the diagram by the presence of *Sphagnum* and *J. articulatus*. The bottom left-hand corner was much drier and bore a dense covering of birch saplings with bracken in the open clearings and a layer of leaf mould.

The distribution of the *Culicoides* larvae found in the samples is given in fig. 10. The most noticeable feature is the concentration of larvae on the right-hand

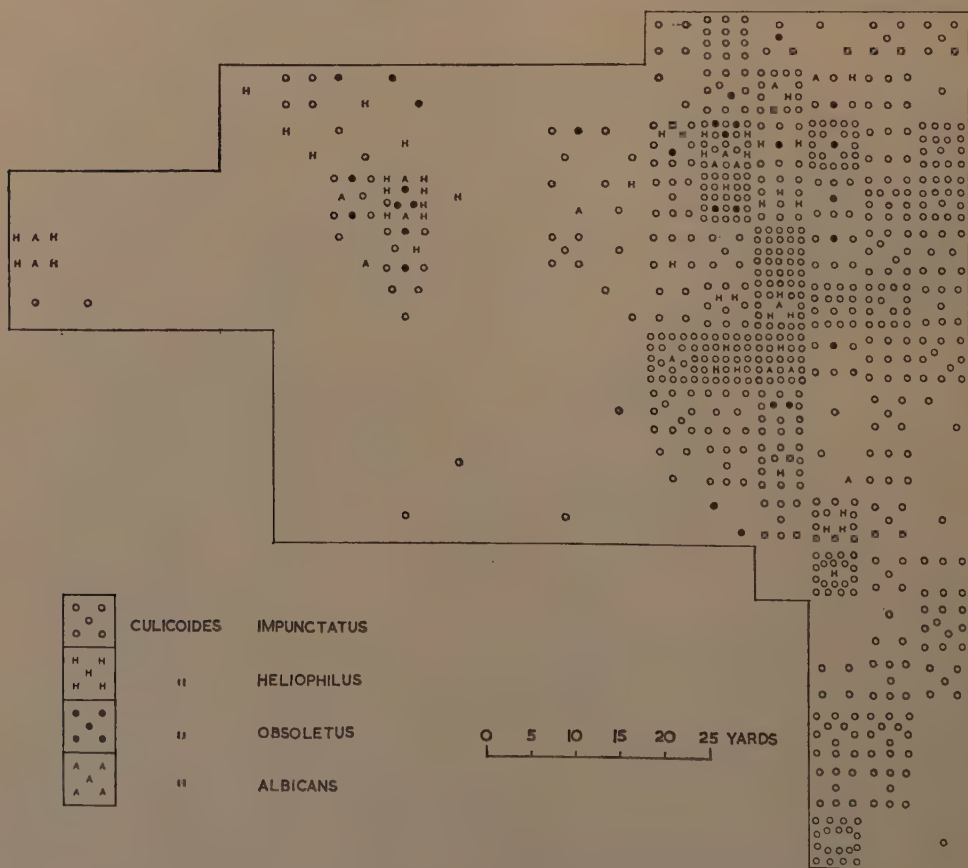


Fig. 10.—Distribution of larvae of *Culicoides* on Bannachra site (cf. fig. 9).

side, where the flow of water down the hillside is unimpeded by the wall. Here larvae of *C. impunctatus* abounded, over 90 per cent. of the total being found in this section, whereas only 42 per cent. of the other three species (*albicans*, *obsoletus* and *heliophilus*) occurred there. These latter species appeared to prefer the wetter and more stagnant environs of the ditch. It will be noted that all species avoided the drier woodland (bottom left-hand corner).

The reason for this dependence of *C. impunctatus* on flowing water is not known, but two suggestions present themselves. Larvae of *Culicoides*, being apneustic, are unable to carry a store of air down into the anaerobic peat. Under these conditions, respiration must present a problem, and flowing water might bring in the essential oxygen. Alternatively, or additionally, the water might bring in food for the larvae. It will be recalled that on Soutra there was evidence of movement of larvae of *C. impunctatus* in response to some oscillating factor, which, it was suggested, might be soil-water level. Only 20–25 per cent. of larvae of *C. impunctatus* produce adults when transferred to 3 × 1 in. tubes containing small pieces of material from the breeding site, although when late fourth-instar larvae of most other species of *Culicoides* (for example, *C. cubitalis*) are kept in this way in the laboratory, about 80 per cent. will successfully complete their development. This relative failure to complete development may be related to the absence of water movement in tubes.

The association between larvae of *C. impunctatus* and vegetation can be carried further by analysing the results from the samples in terms of larval density and plant cover. Nine different plant groupings were recognised and are listed in Table XVII. The analysis of variance was carried out on the transformed data ($\log_{10}(n+1)$) and the result is given below.

Source of variance	Degrees of freedom	Sum of squares	Mean square	F
Between groups	8	6.603395	0.825424	3.50 ***
Within groups	408	39.428386	0.096638	
Total	416	46.031781		

Clearly there are highly significant differences between the groups. In Table XVII, the logarithmic means and their five per cent. fiducial limits are given for each group. Evidently groups 1–5 have significantly higher larval populations than groups 6–9, since the lower fiducial limits of the former exceed the upper fiducial limits of the latter. This is really a division into a wetter zone (groups 1–5) and a drier zone (groups 6–9). Within either zone the various groups do not differ significantly from each other.

These results reinforce the conclusions drawn from the Soutra data that *C. impunctatus* distinguishes between different broad vegetational zones but not between the subdivisions within a zone.

J. articulatus is a useful but not critical indicator plant for breeding sites of *C. impunctatus*. Observations made on many different sites have shown that when it occurs with *Sphagnum*, as at Bannachra, it will support breeding by *C. impunctatus*, but in the absence of *Sphagnum* (as on Soutra, Table VII) it will be associated with freshwater-marsh species, such as *C. cubitalis*.

Other species of Culicoides at Bannachra.

The distribution of the larvae of other species of *Culicoides* at Bannachra is given in Table XVIII in which *C. impunctatus* has been included for comparison. Each of the three species, *C. heliophilus*, *C. obsoletus* and *C. albicans*, is more abundant in the wetter zone I. Although only 56 per cent. of the samples were taken from this zone it provided 90 per cent. or more of the larvae of *C. obsoletus* and *C. heliophilus*, 81 per cent. of *C. impunctatus* and 71 per cent. of *C. albicans*.

TABLE XVII.
Distribution of larvae of *C. impunctatus* among various vegetational groups at Bannachra, 1949.

Zone	Group no.	Vegetation sampled	No. of samples	Total larvae	Arithmetic mean (\bar{n})	Mean $\log_{10}(n+1)$	$t_{0.05} \bar{s} \bar{s}$	Upper or lower 5% fiducial limits	Lower limit	Upper limit
I	1	<i>J. articulatus</i> + 'Gramineae'	20	72	3.60	0.404	± 0.139	0.265	}	}
	2	<i>Sphagnum</i> + 'Gramineae'	23	60	2.61	0.464	± 0.129	0.335		
	3	<i>J. articulatus</i> + <i>Sphagnum</i>	59	134	2.27	0.396	± 0.079	0.317		
	4	'Gramineae'	65	144	2.22	0.348	± 0.077	0.271		
	5	<i>Sphagnum</i>	67	129	1.93	0.331	± 0.075	0.256		
I & II	6	Miscellaneous	76	70	0.92	0.164	± 0.071	0.235	}	}
	7	Bracken	34	23	0.68	0.148	± 0.105	0.253		
II	8	Leaf mould	48	26	0.54	0.106	± 0.089	0.195	}	}
	9	Other mosses	25	7	0.28	0.055	± 0.123	0.178		
		Total	417	665	1.59	0.266				

n = no. of larvae per sample.

The remaining larvae of *C. albicans* were, in fact, taken from samples containing either *J. articulatus* or *Sphagnum* in association with another plant.

C. heliophilus appears to show a preference for *J. articulatus*, but a χ^2 test on the distribution of the larvae of this, *C. obsoletus* and *C. albicans* reveals no significant difference between them, although they differ markedly, collectively, from *C. impunctatus*.

Luss, 1947.

In the autumn of 1947, numerous samples were taken from the vicinity of a small hillside trickle near Luss, Dunbartonshire. Intensive sampling was con-

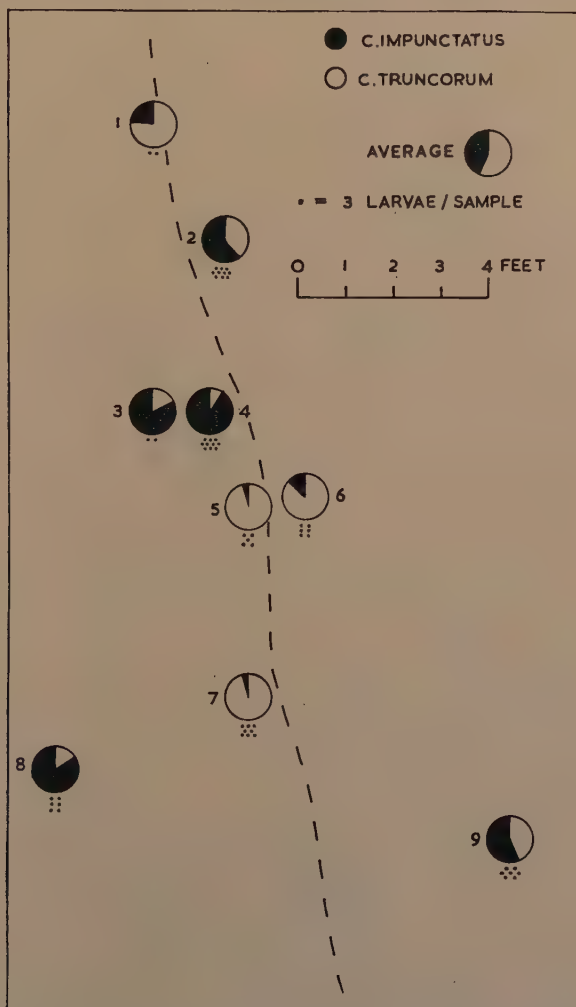


Fig. 11.—Diagrammatic representation of distribution of larvae of *Culicoides* in and near a hillside trickle (broken line) at Luss; relative proportions of *C. impunctatus* (black) and *C. truncorum* (white) given in a circle for each batch of samples. Batch numbers correspond to those in Table XIX, column 1. Each black dot under a circle indicates an average of 3 larvae per sample.

TABLE XVIII.
Distribution of larvae of *Culicoides* between various vegetational groups at Bannachra, 1949.

Zone	Group	Vegetation sampled	No. of samples	%	<i>C. heliophilus</i>		<i>C. obsoletus</i>		<i>C. albicans</i>		<i>C. impunctatus</i>	
					No.	%	No.	%	No.	%	No.	%
I	1	<i>J. articulatus</i> + 'Gramineae'	20	5	9	19	7	25	0	0	72	11
	2	<i>Sphagnum</i> + 'Gramineae'	23	5	3	7	6	21	3	18	60	9
	3	<i>J. articulatus</i> + <i>Sphagnum</i>	59	14	26	55	9	31	8	47	134	20
	4	'Gramineae'	65	16	3	7	1	3	1	6	144	22
	5	<i>Sphagnum</i>	67	16	2	4	3	10	0	0	129	19
I + II	6	Miscellaneous	76	18	4	8	1	3	5	29	70	11
	7	Bracken	34	8	0	0	0	0	0	0	23	3
II	8	Leaf mould	48	12	0	0	2	7	0	0	26	4
	9	Other mosses	25	6	0	0	0	0	0	0	7	1
		Total	417	100	47	100	29	100	17	100	665	100

TABLE XIX.

Plant cover and distribution of larvae of *Culicoides* in a small hillside trickle at Luss, Dunbartonshire, in autumn, 1947. Figures in brackets are percentages of total larvae.

No. on figure	Associated plant species	No. of samples	No. of larvae	Approx. no. larvae per sample	<i>C. impunctatus</i>	<i>C. truncorum</i>	<i>C. albicans</i>	<i>C. heliophilus</i>	<i>C. obsletus</i>
1	<i>Carex riparia</i>	9	56	6	13 (23)	42 (75)	—	1 (2)	—
3	<i>Luzula sylvatica</i>	9	59	7	47 (79)	10 (17)	—	1 (2)	1 (2)
5	<i>Sphagnum auriculatum</i>	9	129	14	5 (4)	123 (95)	—	1 (1)	—
7	<i>canovirens</i>	9	205	23	5 (2)	166 (80)	23 (12)	11 (6)	—
9	<i>Juncus bulbosus</i>	5	105	21	57 (54)	43 (41)	2 (2)	2 (2)	1 (1)
2	<i>Polytrichum commune</i>	3	86	29	52 (61)	32 (37)	—	—	2 (2)
4	<i>Juncus effusus</i>	3	92	31	82 (89)	8 (9)	—	—	2 (2)
6	"	3	54	18	7 (13)	47 (87)	—	—	—
8	"	9	150	17	125 (83)	25 (17)	—	—	—
	Total	59	936	15.9	393 (42.0)	496 (53.0)	25 (2.7)	16 (1.7)	6 (0.6)

ducted over a restricted area (fig. 11), 59 samples being taken from an area of about 10 sq. yd. It was a particularly prolific site, yielding an average of 15.9 larvae per sample, compared with 2.8 on Soutra 1 and 5.4 on Soutra 2.

The larval population was dominated by *C. impunctatus* and *C. truncorum* Edw., which contributed 42 and 53 per cent., respectively; the remaining 5 per cent. was composed of *C. albicans*, *C. heliophilus* and *C. obsoletus*. In spite of *C. impunctatus* and *C. truncorum* having near parity on the over-all figures, fig. 11 shows that groups of samples tended to be dominated by one or the other species. The cause is not certainly known, but seems associated with the water content of the soil. *C. truncorum* was most abundant in samples saturated with water, while *C. impunctatus* showed a preference for the drier, but still decidedly wet, samples. This supports the conclusion drawn from the data from Soutra, where *C. impunctatus* avoided zone II.

C. albicans and *C. heliophilus* were only found in any numbers in the very wet *J. bulbosus* samples, confirming the preference of *C. albicans* for zone II on Soutra 1.

Summary.

A major problem in using larvicides to control *Culicoides* is to recognise the sites requiring treatment. The relationship between plant cover and breeding of *Culicoides* (mainly *C. impunctatus* Goetgh.) was accordingly investigated in moorland areas of Scotland. Two sites were examined on Soutra Hill, Midlothian, and one each on Bannachra Muir and at Luss, Dunbartonshire. Three vegetational zones were recognised on Soutra (site 1): bogland (I), acid grassland (III) and a zone (II) characterised by vegetation preferring a much wetter habitat than either of the foregoing. *C. impunctatus* was virtually the only *Culicoides* species found in zone I; it was relatively less abundant in zone II, where *C. albicans* (Winn.) reached its peak, and occurred most densely at the bogland edge of zone III, where it was associated with *C. cubitalis* Edw., *C. heliophilus* Edw. and *C. obsoletus* (Mg.). Further into the grassland, *C. impunctatus* decreased while the other three species became more numerous and other species, of the group of *C. pulicaris* (L.), appeared. Within each main zone there were no significant differences between the various floristic groups as regards larval densities.

Fluctuations in population density observed in late autumn were regarded as more apparent than real, and attributable to larval movement in response to an oscillating factor, possibly soil-water level. This movement was recognisable in all zones but was most strongly marked where the ground was uneven.

On Soutra (site 2), where only zones I and III were sampled, there was remarkable consistency among the larval densities of the various groups of samples examined. This applied whether the samples were classified on their angiosperm flora, moss cover or spatial arrangement. The main source of variation was within groups and it was found that two adjacent small plots (12 yd. × 12 yd.) which were macroscopically identical showed consistent differences over a period of ten months, covering the transition from one generation to another.

On Bannachra Muir, the larvae of *C. impunctatus* were concentrated in an area covered by *Juncus articulatus* and *Sphagnum* where the water flowed down the hillside near the surface. *C. impunctatus* avoided both an area of drier leaf mould and a stagnant ditch. *C. albicans*, *C. heliophilus* and *C. obsoletus* preferred the ditch to the hillside flow.

Around a hillside trickle at Luss, larvae of *C. impunctatus* and *C. truncorum* Edw. were abundant in subequal numbers. In spite of the restricted size of the area (about 10 sq. yd.) they never occurred in equal numbers in any set of samples, but one or the other predominated. *C. truncorum* showed a preference for the very wet parts.

Acknowledgements.

It gives me pleasure to record my thanks to the bodies that have supported these investigations—the Department of Health for Scotland and the Scottish Tourist Board. I am also indebted to Professor M. M. Swann for giving the Midge Control Unit full use of his department in the University of Edinburgh; to Miss Beryl Leighton, Miss Ruth H. Nash and Mr. D. G. R. Sangster, who collected the basic data; and to Dr. L. J. Hale, with whom much of the statistical analysis was discussed.

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LABORATORY STUDIES ON THE BIOLOGY OF *SYNTOMOSPHYRUM*
ALBICLAVUS KERRICH (HYM., EULOPHIDAE), A PARASITE
 OF TSETSE FLIES.*

By D. S. SAUNDERS †

Department of Zoology, University of Edinburgh.

Waterston (1916) described some female material of *Syntomosphyrum* from puparia† of *Glossina morsitans* Westw. which differed from the typical female of *S. glossinae* Wtstn. in having white-clubbed antennae. The males of these two forms appeared identical, and Waterston considered the specimens to be *S. glossinae*. This 'white-clubbed' form, however, has been shown to be unable to produce biparental offspring when cross-mated with typical specimens of *S. glossinae* (Saunders, 1960a) and has been recognised as a distinct species by Kerrich (1960) and described as *Syntomosphyrum albiclavus*.

On four occasions, the *Syntomosphyrum* parasites of tsetse flies have been used in experimental attempts to control *Glossina* in Africa (Lamborn, 1925; Lloyd, Johnson & Rawson, 1927; Nash, 1933; Lloyd, H. M. (in Swynnerton, 1936)). Although all of these experiments proved unsuccessful, considerable information was collected on methods of rearing the parasites in the laboratory and liberating them in the field. The immature stages of *S. albiclavus* have been described (Saunders, 1960b) and the present paper is a report on the biology of this species.

Apart from the mention of the 'white-clubbed' form of *S. glossinae* by Waterston (1916), the literature gives no indication of the species involved, referring merely to *S. glossinae*. It appears, however, that Lamborn (1916, 1925) was dealing with *S. albiclavus* in his laboratory and field experiments as specimens sent by him to Waterston were the specimens described in Waterston's paper (1916) as the 'white-clubbed' form. All other authors refer to their parasites as *S. glossinae*, but in view of the probably homonymy it is difficult to feel confident of the accuracy of the identification. In the present paper, Lamborn's specimens are referred to as *S. albiclavus*, and all others as *S. glossinae*.

Origin of parasites and method of culture.

The parent material of *S. albiclavus* used in the present investigation emerged from puparia of *G. morsitans* collected near Singida, Tanganyika, and sent to London by air. The parasite readily breeds in blowfly puparia under laboratory conditions (Lamborn, 1925); for this reason the Eulophid was bred in puparia of *Lucilia sericata* (Mg.), thus avoiding the difficulties associated with maintaining a laboratory colony of tsetse flies.

The stock of parasites was kept in 10-in. cubical cages of perspex and terylene gauze at a temperature of about 25°C. in a room in which the relative humidity fluctuated between about 40 and 80 per cent. The parasites were supplied with honey or glucose syrup. Puparia of *Lucilia* were placed on the floor of the cages for about 6 hours daily; during this time they were attacked by the parasites. The parasitised puparia were then removed and incubated at 25°C. until the next

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† Formerly at the Department of Entomology, London School of Hygiene and Tropical Medicine.

‡ To avoid ambiguity in the account that follows, the word 'puparium' will be used to denote the puparial shell and its contents.

generation emerged. Adults not used in experiments were returned to the cages to restock them and, by the methods outlined above, a culture of *S. albiclavus* was established which gave a daily emergence of parasites.

For experimental purposes, temperatures were controlled by the use of incubators and a low-temperature cabinet. Relative humidities were maintained by confining the insects in screw-top kilner jars over sulphuric-acid and water mixtures (Buxton, 1931; Buxton & Mellanby, 1934).

Observations on the biology of *Syntomosphyrum albiclavus*.

The species of fly puparia accepted as host by S. albiclavus.

In the field, the two species of *Syntomosphyrum* (*albiclavus* and *glossinae*) have only been recorded from puparia of tsetse flies (see Saunders, 1960a; Kerrich, 1960), but under laboratory conditions they show no host specificity and have been bred in puparia of 14 other species of Cyclorrhaphous flies. Lamborn (1925) bred *S. albiclavus* (then designated as *S. glossinae*) in puparia of *Musca domestica* *nebulo* F., the Trypetid, *Dacus ciliatus* Lw. (*brevistylus* Bez.), *Chrysomyia putoria* (Wied.) and a species of *Sarcophaga*. Nash (1933) used, as hosts for *Syntomosphyrum glossinae*, puparia of *Chrysomyia marginalis* (Wied.), *C. chloropyga* (Wied.), *C. putoria*, *C. albiceps* (Wied.) and *Sarcophaga haemorrhoidalis* (Fall.), and Roubaud & Colas-Belcour (1936) bred the same parasite in *Musca domestica* L. During the present study, puparia found to be suitable as hosts for *Syntomosphyrum albiclavus* included those of *L. sericata*, *Calliphora vomitoria* (L.), *Phormia regina* (Mg.), *Sarcophaga carnaria* (L.) and *Stomoxys calcitrans* (L.). Puparia of *Drosophila melanogaster* Mg. also proved acceptable, although rather inadequate because of their small size. This range of host puparia, coupled with the fact that only a very small proportion (about 0.25 per cent.) of collected tsetse puparia are parasitised by *Syntomosphyrum*, has led some entomologists (Buxton, 1955) to suggest that these Eulophids are natural parasites of Cyclorrhaphous puparia other than those of *Glossina*.

The condition of the host governing its acceptance.

Nash (1933) claimed that females of *S. glossinae*, under laboratory conditions, could successfully parasitise a freshly-extruded tsetse larva before it had time to burrow beneath the surface of the soil. It has been shown, however, that the immature stages of *Syntomosphyrum* live as ectoparasites in the space between the pupa and the puparium of the host (Saunders, 1960b). Edwards (1954) showed that the presence of this 'sub-puparial space' in a potential host is an important releasing mechanism for oviposition behaviour of the Pteromalid, *Nasonia vitripennis* (Wlk.), which is a Chalcidoid parasite of blowfly pupae very similar in habits to *Syntomosphyrum*. Therefore, Nash's interpretation seems improbable because there is no space beneath the integument of a larva in which the parasite could lay its eggs. Some active larvae of *L. sericata*, which were about to pupate, were offered to some ovipositing females of *S. albiclavus* to see if they could be successfully parasitised. The parasites immediately mounted the larvae but the majority of them were repelled by their activity. Attempts at drilling were not seen. Normal pupation and final emergence of the blowflies followed in due course. In case it was the activity of the larvae which prevented them from becoming parasitised, some prepupae of *Lucilia* in the 'white' stage (less than one hour old) and the 'pale brown' stage (less than three hours old), neither of which contains a sub-puparial space, were also offered to the parasite. In several cases the parasites were observed to push their ovipositors into the prepupae, but after a few days only healthy *Lucilia* adults emerged. From these observations it would appear that the presence of a space beneath the puparial wall in which the eggs can be laid is essential if parasitisation by *Syntomosphyrum* is to be successful.

In view of the fact that Nash (1955) recorded *S. glossinae* breeding in the oöthecae of *Periplaneta americana* (L.) (a record later corrected by Nash in a paper by Jordan (1956) in which the parasite was identified as *Tetrastichus hagenowii* (Ratz.), an endoparasite of cockroach eggs), oöthecae of *Blattella germanica* (L.), and pupae of *Anagasta kühniella* (Zell.), *Ephestia elutella* (Hb.), *Tribolium castaneum* (Hbst.), *Lasioderma serricorne* (F.) and *Stegobium paniceum* (L.) were offered to *S. albiclavus* in addition to fly puparia. Only the oöthecae of the cockroach and the pupae of *Anagasta* and *Ephestia* were mounted, the other pupae being ignored by the parasite. The females rejected the *Blattella* oöthecae after examining the surface with their antennae, but probed with their ovipositors the pupae of *Anagasta* and *Ephestia*; from this it may be assumed that only the latter elicited the correct response from the parasite for 'host selection'. On no occasion, however, did parasitisation result from these attacks upon *Anagasta* and *Ephestia*, presumably because of the absence of the space under the pupal integument.

Pupae do not necessarily have to be alive for *Syntomosphyrum* to breed on them, and the parasites will lay eggs on pupae whatever their condition, so long as there is a space beneath the puparial shell. The larvae are unable to complete their development, however, if the pupa is too dry or in an advanced state of decay. Lamborn (1925) observed that *S. albiclavus* will 'parasitise' dead pupae and produce a second and occasionally a third brood of parasites from a single pupa if there is some unconsumed host material remaining. He (Lamborn, 1916) also recorded this species as a hyperparasite of *Glossina* through *Mutilla glossinae* Turner, another frequent parasite of tsetse flies, and for a long time held the opinion that hyperparasitism was its normal mode of life. In the present investigation it has been found that *S. albiclavus* can produce a brood of offspring from a puparium of *Lucilia sericata* known to be full of diapause larvae of *Nasonia vitripennis* and in which there was none of the original pupal material remaining. These observations make it apparent that *S. albiclavus* is not a parasitoid in the strict sense of the word, but rather a 'refined predator', the female normally killing the pupa before laying her eggs; it can, however, lay eggs in dead hosts, the larvae feeding as scavengers.

The number of adults emerging from the host puparium.

Lamborn (1925) recorded an average of 16 adults of *S. albiclavus* from pupae of *Musca domestica* *nebulosa*; 21 from *Dacus brevistylus*; 67 from *Sarcophaga*; 93 from *Chrysomya* and 34 from *Glossina morsitans*. Nash (1933) recorded 185 adults of *Syntomosphyrum glossinae* from a single pupa of *Sarcophaga haemorrhoidalis*, but this would appear to be a case of superparasitism. Results of a similar investigation during the present work, showing the average size of the

TABLE I.

Number of progeny of *S. albiclavus* emerging from puparia of five species of fly, showing relationship between yield of parasites and weight of host puparium.

Species of host	Range in weight of puparium (mg.)	Mean number of <i>S. albiclavus</i> emerging	Range in numbers emerging	Approx. weight of host material used in the production of each parasite (mg.)
<i>Musca domestica</i>	20-30	29	17-40	0.86
<i>Lucilia sericata</i>	30-40	49	24-71	0.71
<i>Glossina palpalis</i>	30-40	43	31-50	0.81
<i>Calliphora vomitoria</i>	70-80	85	56-157	0.88
<i>Sarcophaga carnaria</i>	120-180	135	100-153	0.92

(R.-D.) brood of *Syntomosphyrum albiclavus* emerging from puparia of *G. palpalis* and four species of blowflies, are shown in Table I. These results show that (if the fly pupae are fully parasitised, without any overcrowding of the developing larvae) the number of parasites which emerge from the puparium is in direct proportion to its size. Large puparia, such as those of *Calliphora* or *Sarcophaga*, may require the eggs of more than one female parasite before becoming parasitised to capacity.

In order to demonstrate more clearly the relationship between the size of the host and the number of parasites developing on it, undersized puparia (of weights between 8.0 and 23.0 mg.) were selected from a starved culture of *Musca domestica*. These puparia were offered to females of *S. albiclavus* for six hours and then were incubated until the offspring emerged. The results (Table II and fig. 1) show a very high correlation between the size of the brood and the weight of the host (correlation coefficient $r = 0.9594$).

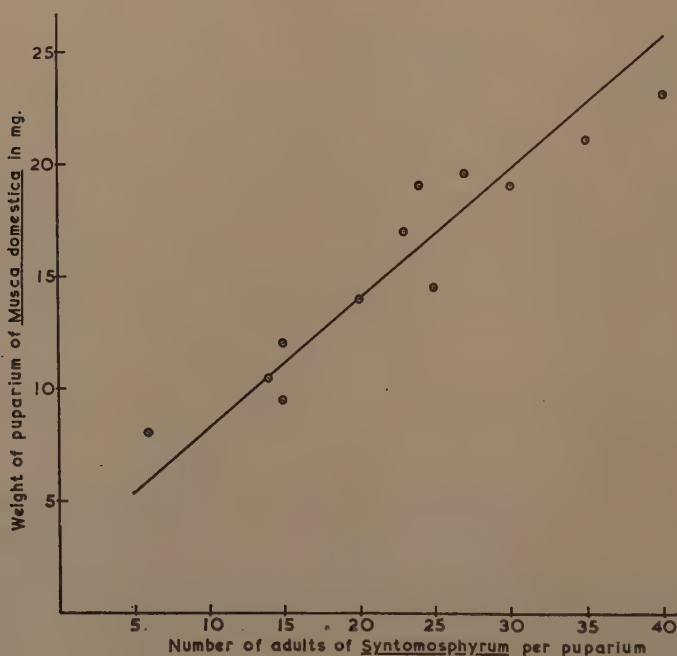


Fig. 1.—The number of adults of *Syntomosphyrum albiclavus* emerging from puparia of *Musca domestica*, showing a positive correlation between the weight of the host puparium and the size of the brood.

(Correlation coefficient $r = 0.9594$.)

The number of offspring produced by a female.

Newly emerged females of *S. albiclavus* were enclosed singly in tubes with a male and a puparium of *L. sericata*. The host puparia were replaced daily and incubated at 25°C. until the progeny emerged. The results (Table III) show that the number of progeny produced may be very variable and in this experiment show a range of 0 to 195 offspring per female. Chorley (1929) conducted a similar experiment and recorded that 1 to 5 male and 60 to 134 female offspring could be

produced by a female of *S. glossinae* during her lifetime. The number of progeny from a female seems to be closely associated with her size, which depends to a large extent upon the degree of crowding of the developing parasites in the host puparium from which she emerged. The four females which failed to produce any offspring were very small and part of a brood from a very overcrowded host.

TABLE II.

The size of the brood of adults of *S. albiclavus* from puparia of *Musca domestica*, showing relationship between yield of parasites and weight of host puparium, and also weight of host material used in the production of each parasite.

Weight of <i>Musca</i> puparium (mg.)	Total number of <i>S. albiclavus</i> emerging	Weight of host material used in the production of each parasite (mg.)
8.0	6	1.33
9.5	15	0.63
10.5	14	0.75
12.0	15	0.80
14.0	20	0.70
14.5	25	0.58
17.0	23	0.74
19.0	24	0.79
19.0	30	0.63
19.5	27	0.72
21.0	35	0.60
23.0	40	0.57

The effect of larval nutrition on fecundity.

In many Hymenoptera, such as the Pteromalid, *Nasonia vitripennis*, the protein obtained during larval life is supplemented by protein obtained by the adults from the fluid which exudes through the hole made in the host during oviposition. This protein has been shown to further egg production (Roubaud, 1917; Flanders, 1935). In *N. vitripennis*, this type of feeding occurs in both sexes, and in the female it has been shown (Edwards, 1954) to be a fixed part of the oviposition behaviour pattern, occurring when the ovipositor is withdrawn from the host tissues. During the first attack by a female upon a host, no eggs are laid and the sole purpose seems to be to obtain a protein meal; eggs are only laid during subsequent attacks. Adults of *S. albiclavus* have been observed, on several occasions, to feed upon the exudates from the host after oviposition, and egg production is probably supplemented by this protein. The phenomenon of 'host feeding', however, is not an integral part of oviposition behaviour in this species and the majority of females leave the host after the withdrawal of the ovipositor, without feeding upon the fluid exuding from the puncture. Females of *Syntomosphyrum* can also lay eggs in the first host attacked without previously having attacked a pupa for the purpose of obtaining some of the host tissue. It is considered, therefore, that the most important source of protein for *S. albiclavus* is that obtained during larval life, and a direct relationship probably exists between the amount of food available to the larvae and the fecundity of the adults, as shown for *Drosophila* (Alpatov, 1932) and *Tineola bisselliella* (Humm.) (Titschack, 1926).

It can be seen from Tables I and II that there is a great variability in the concentration of *S. albiclavus* developing in the host puparium. In Table I, the weight of host material used in the production of each larva varies between

0.71 and 0.92 mg. and in Table II between 0.57 and 1.33 mg. This variation is the direct result of the variable concentration of parasites per host. It is obvious from these figures that in the more crowded hosts each developing parasite has less food material available to it than those developing in less crowded puparia.

In an experiment designed to investigate the effect of larval nutrition on the fecundity of females of *S. albiclavus*, puparia of *L. sericata* were exposed to attack

TABLE III.

The number of offspring produced by a female of *S. albiclavus*.
Each line is concerned with a single female.

Length of life of female (days)	Number of puparia parasitised	Number of offspring
12	0	0
13	0	0
13	0	0
16	0	0
12	4	16
19	4	24
18	6	27
14	3	34
19	4	50
13	6	52
15	6	56
20	7	58
21	3	58
25	5	61
26	9	64
18	5	66
18	4	70
21	5	71
14	7	78
27	13	90
25	9	101
18	6	121
14	7	129
14	6	144
22	6	161
18	8	166
20	7	168
25	10	178
22	12	195

by the parasite for periods varying between three and 24 hours. By this method the number of eggs laid in each puparium, and hence the concentration of parasites, was controlled, the puparia exposed for 3 hours being less heavily parasitised than those exposed for 24 hours. The host puparia were weighed just before and just after the emergence of the parasites; the difference between these two weights gave the weight of pupal material consumed by the larvae. This weight, divided by the number of parasites emerging from the puparium gave the mean weight of host tissue consumed by each parasite. The newly emerged adults of *S. albiclavus* were then kept on a glucose diet at 25°C. and at 80 per cent. relative humidity for three days until maturity, and then fixed in Carnoy's fluid. By this method the insects were prevented from obtaining any protein during adult life which might complicate the experiment. Ten females were selected at random from each batch and their abdominal contents dissected out on a microscope slide into polyvinyl lactophenol in which was dissolved a small amount of lignin pink. This enabled egg-counts to be made and the mean number of eggs per female in each

batch to be calculated. The results of this experiment (Table IV and fig. 2) show that the number of eggs in the ovary after three days is in direct proportion to the weight of host material consumed during larval life (correlation coefficient $r = 0.9796$).

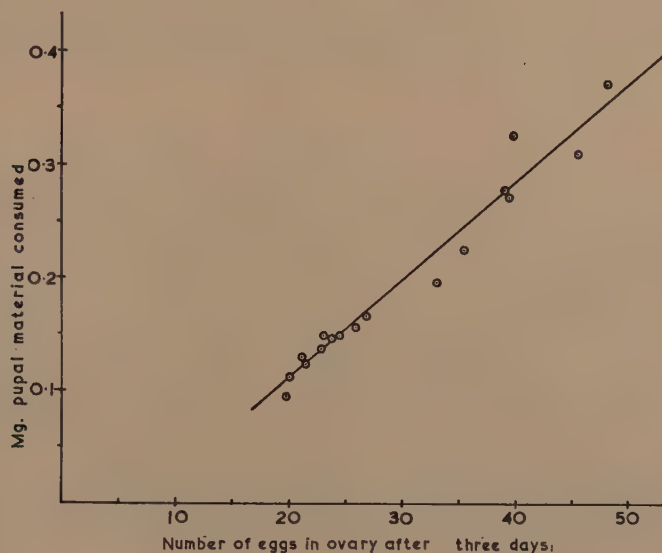


Fig. 2.—The effect of larval nutrition upon the fecundity of females of *Syntomosphyrum albiclavus*, showing that the number of eggs in the ovary after three days is dependent upon the weight of host material consumed during larval life. (Correlation coefficient $r=0.9796$.)

TABLE IV.

The number of fully-developed eggs in the ovaries of females of *S. albiclavus* kept at 25°C. for 3 days, in relation to weight of host material consumed during the larval stage.

Weight of puparium before emergence of parasites (mg.)	Weight of puparium after emergence of parasites (mg.)	Weight of pupal material consumed (mg.)	Number of <i>S. albiclavus</i> emerging	Weight of pupal material consumed per parasite (mg.)	Mean number of eggs per female after 3 days
11.9	6.4	5.5	58	0.09	19.9
14.2	5.5	8.7	77	0.11	20.1
12.6	5.4	7.2	57	0.13	21.4
10.3	6.5	3.8	29	0.13	21.2
20.1	10.6	9.5	69	0.14	22.9
20.3	10.3	10.0	70	0.15	23.8
14.1	6.4	7.7	58	0.15	23.0
19.0	9.5	9.5	63	0.15	24.4
21.4	11.0	10.4	70	0.16	25.9
23.7	11.3	12.4	71	0.17	26.8
21.9	10.6	11.3	54	0.20	33.0
22.6	12.4	10.2	45	0.23	35.4
20.3	12.1	8.2	30	0.27	39.4
16.9	8.2	8.7	31	0.28	39.0
20.4	12.6	7.8	25	0.31	45.5
24.7	12.6	12.1	37	0.33	39.7
25.2	21.1	4.1	11	0.37	48.1

From these results it follows that in a host puparium containing a high concentration of developing parasites there is insufficient food material and the emerging adults have a low fecundity. Conversely, females emerging from puparia which had contained few developing larvae have a high fecundity. The number of eggs which a female of *S. albiclavus* is capable of developing without obtaining additional protein in adult life is, therefore, dependent upon the amount of food available to the developing larvae or the concentration of parasites within the host puparium from which she emerged.

The effect of temperature on the duration of the parasitic phase of the life-cycle.

The only data previously collected for the length of development of *Syntomosphyrum* are those of Nash (1933) who recorded the seasonal variation in the duration of the life-cycle of *S. glossinae* with mean monthly temperatures. In the present investigation the duration of the parasitic phase of *S. albiclavus* was studied at controlled temperatures. In this experiment, puparia of *L. sericata* and *Musca domestica* were exposed to the parasites for three hours, placed in a relative humidity of 80 per cent. and incubated at a series of temperatures between 18 and 35°C. The date of emergence of the next generation of parasites was then noted. It normally took several days for the emergence of the parasites to be completed, especially at the lower temperatures, in which the duration of development was much protracted. For the purposes of this experiment, however, the duration of the parasitic phase was defined as the time between the insertion of the eggs into the host puparium and the emergence of 50 per cent. of the progeny. At the higher temperatures, 50 per cent. emerged within a few hours of the appearance of the first parasite.

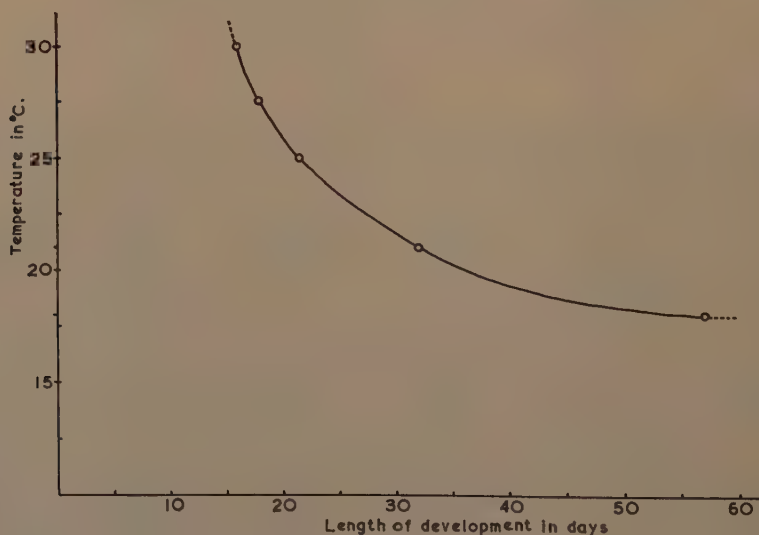


Fig. 3.—The effect of temperature upon the duration of the parasitic phase in the life-cycle of *Syntomosphyrum albiclavus*.

The results of this experiment (Table V) show that the duration of the parasitic phase is dependent upon the temperature, being shorter at higher temperatures than at lower. The result of plotting the temperature against the duration of development (fig. 3) shows an almost typical temperature hyperbola. In most

graphs of this type, as shown by Ludwig & Cable (1933) for the development of *Drosophila* pupae, and by Melvin (1934) for the development of *Phormia* eggs, the duration of development increases again at high temperatures after reaching its shortest (the optimum) point. In the case of *S. albiclavus*, however, there is no such change; apparently the effects of this increase are masked by a mortality of the immature stages at temperatures above 32.5°C.

TABLE V.

The duration of the parasitic phase of *S. albiclavus*,
in relation to temperature.

Temperature (°C.)	Number of host puparia used	Duration of the parasitic phase in days
35.0	50	—*
32.5	100	—
30.0	140	16
27.5	100	18
25.0	120	21-22
21.0	100	32
18.0	60	57

* No parasites completed their development at 32.5°C. or above.

The thermal death point of the immature stages of *S. albiclavus* is somewhere between 30 and 32.5°C. (Table V). In order to ascertain the stage in the life-cycle which is susceptible to high temperatures, eggs, larvae and pupae were incubated at 35°C. The duration of each developmental stage was first established by a daily examination of the contents of parasitised *Lucilia* puparia incubated at 25°C. (Table VI). Host puparia containing eggs of *S. albiclavus* were then exposed to a temperature of 35°C. for 24 hours and then incubated at 25°C. until the parasites emerged. Puparia of *Lucilia* previously kept at 25°C. were exposed to 35°C. from the 6th to the 10th day, when they were estimated to contain fourth-instar larvae, and then returned to 25°C. to complete their development. Similarly, host puparia were exposed to 35°C. whilst containing pupae of the parasite. Batches of control hosts, parasitised at the same time and under identical conditions as the experimental puparia, were used in each case and kept throughout at 25°C. The results

TABLE VI.

The duration of each stage of *S. albiclavus* at 25°C.

Stage	Duration in days
Ovum	2
Larva I	1
Larva II	1
Larva III	1
Larva IV	5
Prepupa	2
Pupa	8-9
Adult within host puparium before emergence	1
Total	21-22 days

(Table VII) show that no significant mortality occurred in those parasites exposed to 35°C. as larvae or pupae but a high mortality occurred to those exposed as eggs. On dissection, the host puparia so exposed when the parasite was in the egg stage showed no remains of dead larvae or pupae of the Eulophid, so it appears that a constant temperature of 35°C. is lethal to the eggs of *S. albiclavus*.

TABLE VII.

The effect of exposure to high temperature upon the immature stages of *S. albiclavus*.

Stage at which exposed to 35°C.	Experimental puparia			Control puparia (25°C.)		
	Number of puparia parasitised	Number of parasites emerging	Number of parasites per host	Number of puparia parasitised	Number of parasites emerging	Number of parasites per host
Eggs : 0-1 day ..	3	11	3.7	7	185	26.4
4th-instar larvae : 6-10 days ..	4	103	25.7	5	135	27.0
Pupae : 12 days- emergence	7	274	39.1	6	234	39.0

The survival of adults of Syntomosphyrum albiclavus at various combinations of temperature and humidity.

Freshly emerged adults of *S. albiclavus* were placed in small glass tubes fitted with nylon gauze tops, and exposed to a series of temperatures between 15 and 35°C. and humidities between 20 and 100 per cent. Few males were available for this experiment because the progeny of fertilised females consists largely of females. Generally, 20 females and as many males as were available were placed in each tube and five replicates of each were incubated at each combination of temperature and humidity. Each result for the females, therefore, is based upon 100 insects, but those for the males upon a smaller number. The contents of the tubes were examined after 24 hours, four days, seven days and then at weekly intervals, and the percentage mortality of the parasites was recorded. In view of the fact that parasites from overcrowded hosts are small and weakly, the size of those used in this experiment was controlled by selecting individuals only from host puparia which had contained a moderate number of developing parasites.

The results (Table VIII) show that the percentage mortality is greater at higher temperatures and that the males do not live so long as the females. At each temperature tested (fig. 4) humidity has a similar effect upon survival. Mortality is greatest at low humidities, the parasites dying from desiccation, and the optimum relative humidity for the females at all temperatures tested is about 80 per cent. This result is similar to that obtained by van der Merwe (1943) for the Pteromalid, *N. vitripennis*. In saturated conditions there is an increased mortality.

In experiments of 24 hours or more, most insects, unless they are able to resist desiccation (e.g., *Tenebrio* and adult fleas (Mellanby, 1932)), show a greater survival at high than at low humidities. Examples of this are furnished by the larvae of *Popillia japonica* Newm. (Ludwig & Landsman, 1937) and adults of

Pediculus and *Lucilia* (Mellanby, 1932). From the present results, therefore, it is apparent that females of *S. albiclavus* are unable to resist desiccation and die rapidly in dry air. There are few examples of an increased mortality at very high humidities; among these, Leeson (1932), working with starved adult fleas, found that there was an optimum for survival at about 90 per cent. humidity and a somewhat increased mortality in saturated air. In the present experiments, death of

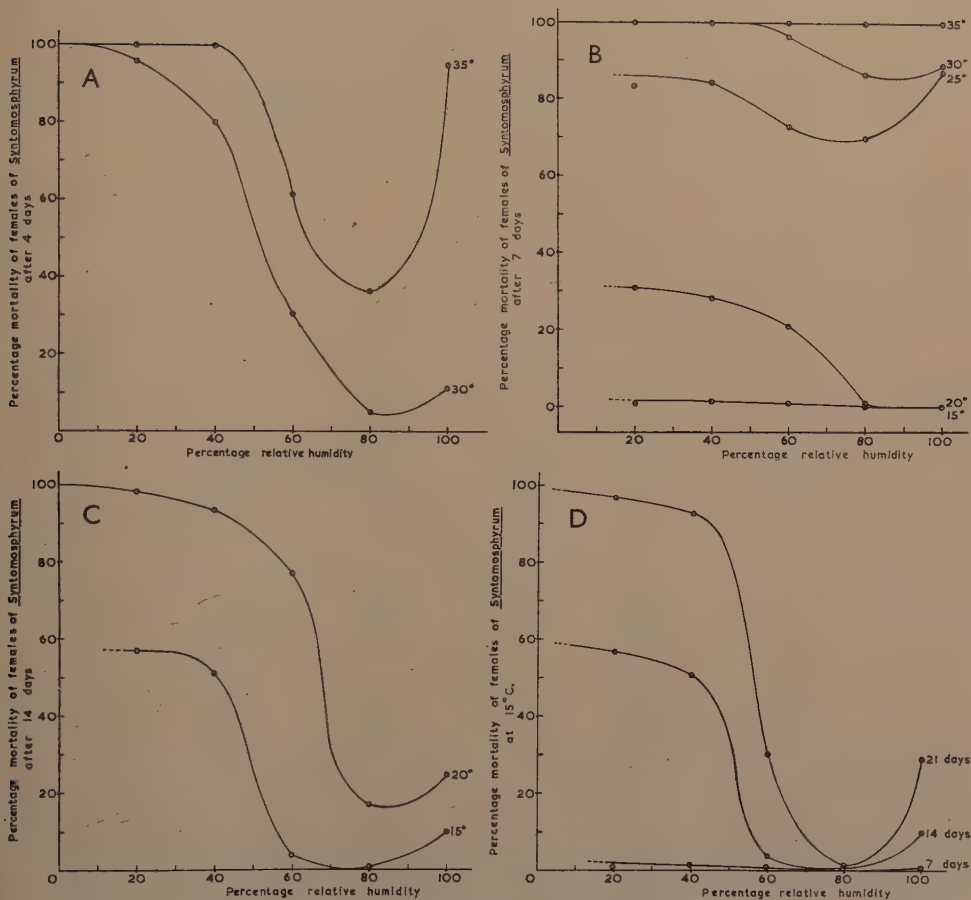


Fig. 4a-d.—The mortality of adult females of *Syntomosphyrum albiclavus* at various combinations of temperature (°C.) and relative humidity: (a) after four days at high temperatures; (b) after seven days at all temperatures tested; and (c) after 14 days at low temperatures. These graphs show a high mortality in low humidities, especially in the higher temperatures, an optimum for survival at about 80 per cent. R.H., and an increased mortality in saturated air. Fig. 4d shows the percentage mortality of females of *S. albiclavus* after 7, 14 and 21 days at a low temperature (15°C.).

S. albiclavus at 100 per cent. humidity was probably caused by the parasites becoming trapped in the drops of water which condense on the sides of the tubes and accelerated by the growth of moulds which rapidly develop at high humidities.

The effect of temperature upon ovarian development.

Each ovary of *S. albiclavus* consists of three polytrophic ovarioles which, in a newly emerged female, do not contain fully developed eggs. As the eggs mature they are ovulated and accumulate at the posterior end of the ovary. In a gravid female, up to 25 or 30 fully developed eggs (of length between 0.41 and 0.43 mm.) may be present in each ovary. Oviposition can occur as soon as mature eggs have been ovulated from the ovaries, but females normally attack a host when their

TABLE VIII.

The percentage mortality of males and females of *S. albiclavus* in a range of different temperatures and relative humidities, showing an optimum for survival at about 80 per cent. R.H. and a high mortality in dry and saturated conditions.

Females						
Temp. (°C.)	Day	Percentage relative humidity				
		20	40	60	80	100
35	1	0.0	0.0	0.0	0.0	0.0
	4	100.0	100.0	61.7	36.3	95.4
	7	100.0	100.0	100.0	100.0	100.0
30	1	0.0	0.0	0.0	0.0	0.0
	4	96.0	80.1	30.7	5.7	11.5
	7	100.0	100.0	96.4	86.4	88.9
25	4	9.2	18.1	5.0	11.0	21.0
	7	83.6	84.1	73.1	70.0	89.0
	14	100.0	100.0	100.0	100.0	100.0
20	4	0.0	0.0	0.0	0.0	0.0
	7	30.9	28.2	21.4	1.1	0.0
	14	98.3	93.8	76.5	17.2	24.7
15	7	1.0	1.9	1.0	0.0	1.0
	14	57.0	50.9	4.0	0.9	10.0
	21	97.0	93.1	30.2	1.9	29.1
Males						
35	1	94.2	65.8	0.0	0.0	0.0
	4	100.0	100.0	100.0	100.0	100.0
30	1	66.6	11.8	0.0	0.0	0.0
	4	100.0	100.0	100.0	82.4	90.0
	7	100.0	100.0	100.0	100.0	100.0
25	4	87.0	100.0	100.0	100.0	100.0
	7	100.0	100.0	100.0	100.0	100.0
20	4	81.3	91.3	51.0	20.7	0.0
	7	100.0	100.0	100.0	95.0	59.1
	14	100.0	100.0	100.0	100.0	100.0
15	7	90.0	73.5	27.1	0.0	4.6
	14	100.0	100.0	95.5	77.6	70.5
	21	100.0	100.0	100.0	100.0	100.0

ovaries are full of eggs, and a batch of as many as 50 eggs may be deposited by a single female in a puparium.

In order to investigate the effect of temperature upon the maturation of the ovaries, batches of freshly emerged females of *S. albiclavus* were incubated in a relative humidity of 75 per cent. at 18, 21 or 30°C. At intervals, samples of 10 females were taken at random from each batch and dissected in polyvinyl lactophenol on a microscope slide. The length of the largest egg and also the number of fully developed eggs present were noted. A batch of females was considered 'mature' when a steady number of fully developed eggs was found in the ovaries of the sample females on consecutive days; this was found to agree closely with the age at which parasites attacked host puparia. It will be seen that the number of eggs developed by females in each of the three batches varied considerably. This is correlated with the concentration of parasites developing in the host from which the batch of females emerged.

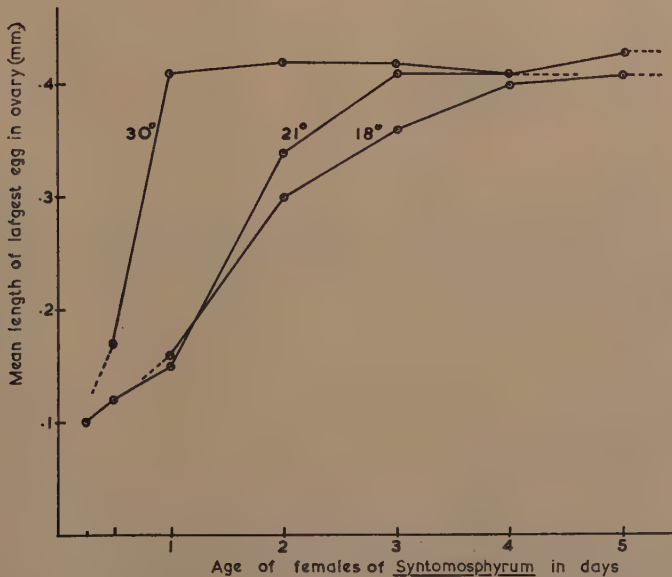


Fig. 5.—The effect of temperature (in °C.) upon ovarian development in *Syntomosphyrum albiclavus*, showing that development (measured as the size of the largest egg in the ovary) is more rapid at higher temperatures.

The results (Table IX and fig. 5) show that at 30°C. maturity is reached after 1 to 2 days, at 21°C. after 4 to 5 days and at 18°C. some time after the 5th day. From these results it follows that the rate of maturation of the ovaries depends upon the temperature, a more rapid development occurring at 30 than at 18°C.

A few females of *S. albiclavus* were incubated at 10°C. These developed very few eggs even after 50 days and were too lethargic to attack the *Lucilia* puparia provided. Consequently, 10°C. must be too low a temperature for this parasite to breed.

The daily production of offspring of 60 females of *S. albiclavus* is shown in Table X and fig. 6. In this experiment, 60 newly emerged females were incubated at 25°C. in separate tubes and supplied with *Lucilia* puparia, which were replaced daily and kept at 25°C. until the emergence of the next generation. It can be

TABLE IX.

The length of the largest egg present and the number of fully developed eggs present in the ovaries of females of *S. albiclavus* at three temperatures.

Age of female (days)	Temperature					
	30°C.		21°C.		18°C.	
	Number of fully developed eggs	Mean length of largest egg (mm.)	Number of fully developed eggs	Mean length of largest egg (mm.)	Number of fully developed eggs	Mean length of largest egg (mm.)
$\frac{1}{2}$	—	—	0	0.10	—	—
$\frac{3}{2}$	0	0.17	0	0.12	—	—
1	17	0.41	0	0.15	0	0.16
2	35.1	0.42	3.1	0.34	2.3	0.30
3	34.8	0.42	15.1	0.41	5.4	0.36
4	34.0	0.41	18.7	0.41	12.7	0.40
5	—	—	20.3	0.43	13.0	0.41
7	—	—	20.5	0.42	—	—

seen that eggs were not laid until the third day after emergence, when the parasites reach maturity, and the daily production of offspring gradually fell off with increasing age of the females. Very old females, although attacking host puparia, were unable to parasitise them.

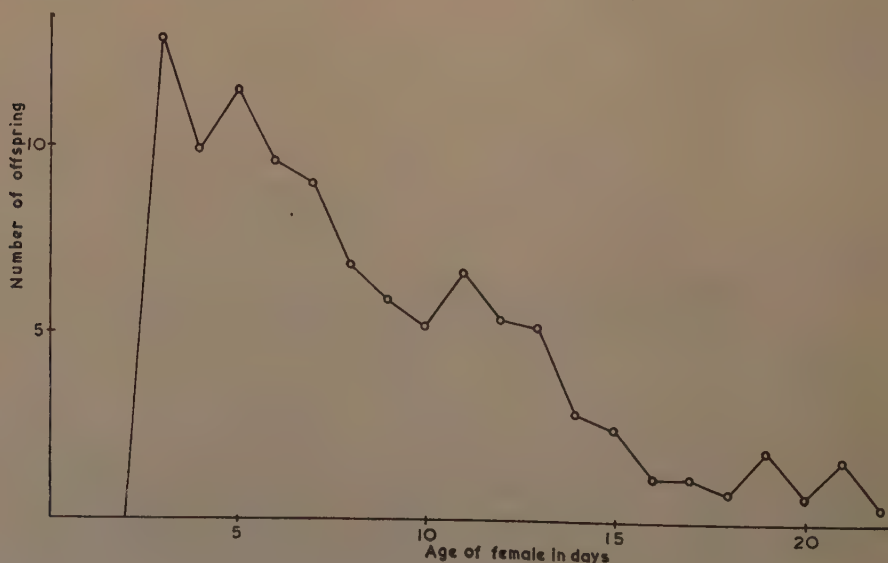


Fig. 6.—The mean number of offspring produced by 60 females of *Syntomosphyrum albiclavus*, showing that no offspring are produced until the third day after emergence and the number produced falls off with the increasing age of the females.

Summary.

Laboratory studies are reported on *Syntomosphyrum albiclavus* Kerrich, a Eulophid of interest as a pupal parasite of tsetse flies. Material for a laboratory culture was obtained from puparia of *Glossina morsitans* Westw. collected in Tanganyika and sent to London by air.

TABLE X.

Relationship of productivity of females of *S. albiclavus* (at 25°C.) to age.

Age of females (days)	Number of females of <i>S. albiclavus</i> alive	Total number of offspring produced	Mean number of offspring per female
1	60	0	0
2	60	0	0
3	60	776	12.9
4	60	596	9.9
5	60	692	11.5
6	60	579	9.6
7	59	532	9.0
8	58	394	6.8
9	58	342	5.9
10	55	284	5.2
11	49	323	6.6
12	47	253	5.4
13	39	204	5.2
14	33	96	2.9
15	27	68	2.5
16	24	28	1.2
17	24	28	1.2
18	18	14	0.8
19	13	25	1.9
20	11	8	0.7
21	9	15	1.7
22	7	3	0.4
23	3	13	4.3
24	3	1	0.3
25	2	0	0.0
26	1	2	2.0

'Natural' and 'unnatural' hosts of this parasite were investigated. *S. albiclavus* parasitised all species of Cyclorrhaphous puparia offered in the laboratory, but has only been recorded from puparia of *Glossina* under natural conditions. The conditions which make a host suitable for *S. albiclavus* are discussed. The most important of these is the presence of the 'sub-puparial space' in which the eggs are laid, without which the host is unacceptable. *S. albiclavus* will also breed on dead pupae and in puparia containing other parasites.

The number of parasites emerging from the host puparium is shown to be directly proportional to its weight.

The fecundity of *S. albiclavus* is shown to be very variable and dependent upon the weight of host material consumed during larval life, which, in turn, reflects the density of parasites in the host puparium from which the females emerged.

The duration of the parasitic phase of the life-cycle and the length of life of the adults are shown to be functions of the temperature. Length of life is also affected by the relative humidity, increased mortality occurring in low humidities and in saturated air, with an optimum for survival at about 80 per cent. R.H.

It is shown that a constant temperature of 35°C. is fatal to the eggs of *S. albiclavus*.

The age at which females of *S. albiclavus* become mature was investigated at three temperatures and it is shown that the rate of maturation of the ovaries is a function of the temperature.

At 25°C., females of *S. albiclavus* are unable to lay eggs until the third day after emergence. The number of offspring produced then falls off with increasing age of the female and very old females, although attacking hosts, are unable to oviposit.

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AN ASSESSMENT OF THE ECONOMIC IMPORTANCE OF THE TSETSE SPECIES OF SOUTHERN NIGERIA AND THE SOUTHERN CAMEROONS BASED ON THEIR TRYPANOSOME INFECTION RATES AND ECOLOGY.

By A. M. JORDAN

*West African Institute for Trypanosomiasis Research,
Kaduna, N. Nigeria.*

Several workers have recorded trypanosome infection rates for various species of *Glossina* which occur in southern Nigeria. This paper is intended both as a review of the literature and as a medium for the presentation of further data; following this, the economic importance of the various species is assessed.

The term "southern Nigeria" is defined as including all areas south of the Southern Guinea Savannah vegetation zone. Tsetse populations have been investigated in Derived Savannah (savannah derived from the original rain-forest of which only vestiges remain), Lowland Rain Forest and Mangrove Forest and Coastal Vegetation. The names of all vegetation zones have been taken from Keay (1953).

All data on infection rates include only mature infections, unless otherwise stated. Trypanosome infections have been classed as being of the groups of *Trypanosoma vivax*, *T. congolense* or *T. brucei*, respectively, according to whether parasites were present in the hypopharynx only, hypopharynx and gut or in the salivary glands of the fly; morphological identifications were not made.

Infection rates of tsetse in the area studied.

Infection-rate data for the tsetse species of southern Nigeria and the Southern Cameroons, from all sources combined, are given in Table I. Infections of the *vivax* group predominated; the proportion of trypanosomes of the *congolense* group was highest in *G. tabaniformis* Westw. A more detailed analysis of the trypanosome infection rates in the various species of *Glossina* follows.

TABLE I.

Trypanosome infection rates: combined data from all sources.

Species	Total dissected	Total positive		Analysis of infections		
		No.	%	<i>T. vivax</i> (%)	<i>T. congo-lense</i> (%)	<i>T. brucei</i> (%)
<i>G. palpalis</i>	2,497	45	1.8	75.6	24.4	0
<i>G. caliginea</i>	230 ¹	84	36.5	84.5	14.3	1.2
<i>G. pallicera</i>	119	3	2.5	(3) ²	(0)	(0)
<i>G. longipalpis</i>	4,360 ³	939	21.5	82.0	18.1	0.1
<i>G. medicorum</i>	252	39	15.5	87.2	12.8	0
<i>G. fusca</i>	1,301	206	15.8	88.8	10.7	0.5
<i>G. tabaniformis</i>	3,389	110	3.2	60.9	38.2	0.9
<i>G. haningtoni</i>	59	5	8.5	(5) ²	(0)	(0)
<i>G. nigrofusca</i>	182	44	24.2	88.6	11.4	0

Note 1. Includes 223 dissected from the Cameroon Republic.

2. Actual numbers.

3. Males only.

G. palpalis (R.-D.).

This species is widespread in southern Nigeria and in the Cameroons, but often in low density. Its habitat is not so strictly riverine as it is in the drier northern part of Nigeria; in the wet season, *G. palpalis* may be found far from streams and occurs in small numbers, throughout the year, even in the rain-forest.

Of the 2,497 dissections referred to in Table I, 1,635 are from Page (1959*b*), whose collections were made at the West African Institute for Trypanosomiasis Research (W.A.I.T.R.) Field Station at Ugbobigha in Benin Province, Western Region; a further 862 dissections were carried out by the author and other workers of W.A.I.T.R. at nine other localities, given in Table II and shown in fig. 1. Data from area A (N. Oyo Province) are included, although this area is in the Guinea Savannah vegetation zone north of the Derived Savannah zone.

TABLE II.

Infection rate in *G. palpalis*.

Area (see fig. 1)	Locality	Date	No. dissected	Total positive	
				No.	%
A	N. Oyo Province	Feb./Mar. 1960	109	2	1.8
B	Upper Ogun Estate	Feb. 1960	208	0	0
C	Fashola Stock Farm	Jan. 1949/May 1958	153	0	0
D	W. Ibadan Province	Mar. 1960	78	0	0
E	Badagri	Mar./Apr. 1960	60	1	1.7
F	Ado Ekiti	Oct. 1956/May 1957	47	3	6.4
G	Ezangbo V.I.C.	Oct. 1959	27	2	7.4
H	S. Cameroons (forest belt) ..	Nov./Dec. 1959	59	0	0
I	S. Cameroons (mangrove swamps)	Nov. 1959	121	3	2.5
Total			862	11	1.3

A study of all the data suggests that in the southern part of Nigeria and the Cameroons the infection rate in *G. palpalis* was normally very low, 1-2 per cent., and that two-thirds or more of the infections belonged to the *vivax* group. Males and females were equally infected. In southern Nigeria and the Cameroons, *G. palpalis* fed largely on man and reptiles (Jordan, Lee-Jones & Weitz, 1961) and these hosts are unimportant as reservoirs of animal trypanosomiasis. The low proportion of flies infected with trypanosomes pathogenic to animals is probably due to the small number of meals derived from important natural reservoirs of such trypanosomes.

These findings are similar to those of Nash & Page (1953), who worked in northern Nigeria; they obtained an infection rate of 3.3 per cent. from 3,382 males of *G. palpalis*, and found that 91 per cent. of the infections belonged to the *vivax* group.

G. tachinoides Westw.

This species has not been studied at the southern limit of its range in Nigeria and no infection-rate data are available. Nash (1948) summarised information obtained elsewhere.

G. caliginea Aust.

This species has a very limited distribution along the West African coast and is generally associated with mangroves and swamp forest. Only seven specimens

have been dissected from the British Cameroons (all negative), but Roubaud, Maillot & Rageau (1951) found heavy infections in *G. caliginea* near Douala, in the Cameroon Republic, some of which may have matured after capture as the flies were taken to Paris for dissection. Of 223 flies dissected, 84 (37.7 per cent.) were infected; 218 of the flies were males.



Fig. 1.—Map of southern Nigeria and the Southern Cameroons, showing areas from which samples of *Glossina* species were dissected.

G. pallicera Big.

This species lives in rain-forest and little is known of its habits. There would appear to be nothing in the literature concerning its ability to transmit trypanosomiasis.

G. pallicera occurs in small numbers at Ugbobigha (fig. 1); between 1955 and 1959, 55 specimens were dissected and two (4 per cent.) were infected with trypanosomes of the *vivax* group. *G. pallicera* is widespread in the forest areas of the Southern Cameroons; during a tour of these areas in November–December 1959, 64 specimens were dissected and one (2 per cent.) had an infection of the *vivax* group.

G. longipalpis Wied.

In southern Nigeria, *G. longipalpis* occurs mainly in Derived Savannah north of the rain-forest belt. The results of the 4,360 dissections referred to in Table I have been taken from Page (1959a); all specimens were males, collected at Ugbobigha between 1954 and 1957. Most (82 per cent.) of the infections were of the *vivax* group. No further records are available from southern Nigeria, but at Takoradi, Ghana, Morris (1934) found infection rates (most of which were of the *congolense* group) ranging from 24 to 29 per cent. during the early rains; in Portuguese Guinea, Fontoura de Sequeira (1935) found an infection rate of 35.3

per cent. among 400 examples of *G. longipalpis* collected during the wet season. Immature infections may have been included by the last two authorities.

From the evidence available, *G. longipalpis* would appear to be a heavily infected species. Jordan, Lee-Jones & Weitz (1961) found that this species fed mainly on Bovidae (95 per cent. of meals identified from Ugobigha), especially bushbuck (*Tragelaphus scriptus*); the importance of these hosts may account for the large number of infections of the *vivax* and *congolense* groups.

G. medicorum Aust.

As yet, this species has been recorded from only three localities in Nigeria; in common with other species of the group of *G. fusca* (Wlk.), its distribution is now discontinuous, as the forest belt has become fragmented by encroaching cultivations. In Nigeria, *G. medicorum* frequents relic forest in Derived Savannah, often together with *G. fusca*, but the latter species also occurs within the rain-forest.

Of the records of 252 dissections of *G. medicorum* referred to in Table I, 50 are from Nash (1952), who collected his specimens from the Olokemeji Forest Reserve, near Ibadan, Western Region (see fig. 1), and 91 are from Page (1959c), who collected his material at Ugobigha from 1955 to 1957. A further 111 dissections were carried out by the author at both localities from 1957 to 1959, continuously at Ugobigha and during two short visits to Olokemeji, one in the wet and one in the dry season. The total figures for each area are compared in Table III.

TABLE III.
Infection rate in *G. medicorum*.

	No. dissected	Ugobigha			
		Total positive		Analysis of infections	
		No.	%	<i>T. vivax</i> (no.)	<i>T. congolense</i> (no.)
Males	75	8	10.7	8	0
Females	58	13	22.4	12	1
Total	133	21	15.8	20	1
Olokemeji					
Males	63	8	12.7	7	1
Females	56	10	17.9	7	3
Total	119	18	15.1	14	4

Infection rates, at 15-16 per cent., were similar in both areas; Jordan, Lee-Jones & Weitz (1961) showed that the natural hosts of *G. medicorum* were also similar in both localities. Bovidae provided more than 70 per cent. of the meals, with bushbuck as the most important single host; perhaps a similar natural reservoir of trypanosomes was available in both areas. The differences between the proportion of infected males and infected females in the small samples from both Ugobigha and Olokemeji are not significant by the χ^2 test, but, in both areas, there is a suggestion that larger numbers would have shown a greater proportion of females to have been infected.

G. fusca (Wlk.).

In southern Nigeria and the Cameroons, *G. fusca* can occur in a wider range of habitats than other species of the *fusca* group. It may be found in riverine forest and forest islands in the savannah, as well as in rain-forest.

Of the 1,301 dissections of *G. fusca* referred to in Table I, 579 are from Page (1959c), who collected his material at Ugbobigha from 1955 to 1957. Dissections were continued at Ugbobigha from 1957 to 1959 by the author, who also dissected specimens collected from Olokemeji during two short visits, one in the wet and one in the dry season; the results from Olokemeji showed no significant difference according to the time of year the flies were collected. The total figures for each area are compared in Table IV.

TABLE IV.

Infection rate in *G. fusca*.

	Ugbobigha				
	No. dissected	Total positive		Analysis of infections	
		No.	%	<i>T. vivax</i> (no.)	<i>T. congolense</i> (no.)
Males	486	51*	10.5	39	11
Females	348	60	17.2	49	11
Total	834	111*	13.3	88	22
	Olokemeji				
	No. dissected	Total positive		Analysis of infections	
		No.	%	<i>T. vivax</i> (no.)	<i>T. congolense</i> (no.)
Males	251	23	9.2	23	0
Females	216	72	33.3	72	0
Total	467	95	20.3	95	0

*Includes one infection of the *brucei* group.

The infection rate was 13.3 per cent. at Ugbobigha and 20.3 per cent. at Olokemeji and there is a significant difference between the proportion of infected flies found at the two localities ($\chi^2=10.59$; $P<0.01$),* which is due entirely to the large difference between the proportion of infected females in the two areas ($\chi^2=18.36$; $P<0.001$). The difference in male infection rates is not significant ($\chi^2=0.19$; $P>0.5<0.7$). The reason for the larger proportion of infected females at Olokemeji is uncertain but, unlike *G. medicorum* referred to above, *G. fusca* derived its main food supply from different sources in the two areas; Jordan, Lee-Jones & Weitz (1961) found that, at Ugbobigha, 73 per cent. of the meals were taken from red river hog (*Potamochoerus porcus*), whereas, at Olokemeji, 80 per cent. of meals were from Bovidae, mainly bushbuck. It would, therefore, not be surprising if the flies acquired different degrees of infection in the two areas; why such a difference only occurred among the females is unknown. One cannot postulate that, unlike the males, the females selected different host species in each area, because Jordan, Lee-Jones & Weitz (1961) showed that the diet of both sexes was similar within each area; no satisfactory explanation can be produced.

* Yates' correction has been applied to all Chi-square calculations in this paper.

In both areas a higher proportion of females than males was infected (Ugbobigha: $\chi^2=7.43$; $P<0.01$; Olokemeji: $\chi^2=40.38$; $P<0.001$). This is in accordance with evidence, to be presented elsewhere, that females of *G. fusca* are longer-lived than males.

No infections of the *congolense* group were found at Olokemeji, but at Ugbobigha 22 of 111 (19.8 per cent.) infections belonged to this group.

G. tabaniformis Westw.

In Nigeria this species occurs in the wetter areas of rain-forest and is widespread throughout this belt in the Southern Cameroons.

Of the 3,389 dissections of *G. tabaniformis* referred to in Table I, 2,380 are from Page (1959c), who collected his material at Ugbobigha from 1955 to 1957; the author, also working at Ugbobigha, added a further 980 dissections from 1957 to 1959 and another 29 from material collected in the Southern Cameroons, which will be referred to separately. Of the 3,360 specimens of *G. tabaniformis* collected at Ugbobigha, 3.2 per cent. were infected. There was a significantly higher infection rate amongst females (84 of 1,975 flies dissected, or 4.3 per cent.) than amongst males (24 of 1,385 flies dissected, or 1.7 per cent.): $\chi^2=15.82$; $P<0.001$. There was a tendency for infections of the *congolense* group to predominate in males (11 of *vivax*, 13 of *congolense*) and infections of the *vivax* group in females (54 of *vivax*, 29 of *congolense*), but the difference is not significant ($\chi^2=2.14$; $P>0.1<0.2$).

The proportion of *G. fusca* infected at Ugbobigha was significantly higher than the proportion of *G. tabaniformis* infected ($\chi^2=135.55$; $P<0.001$), both species having been collected from the same area of rain-forest. This may suggest that *G. fusca* is more readily infected than *G. tabaniformis*, and this possibility requires investigation, but it is perhaps more likely to be a result of differences in the feeding habits of the two species. In the same area of forest both tsetse fed mainly on red river hog (*G. tabaniformis*, 72 per cent. of meals, *G. fusca*, 73 per cent.), but, whereas 21 per cent. of the meals of *G. tabaniformis* were from porcupine (*Atherurus africanus*), 21 per cent. of those of *G. fusca* were from Bovidae (Jordan, Lee-Jones & Weitz, 1961). The difference in the proportions of porcupine and Bovid blood-meals taken by the two species were significant and it is possible, as Bovidae are important natural reservoirs of trypanosomes, that this may account for the higher infection rate observed in *G. fusca*. There is no evidence for greater longevity of *G. fusca* which, if it occurred, might have accounted in part for a higher infection rate in that species.

A further 29 specimens of *G. tabaniformis*, collected in rain-forest between Mamfe and Victoria in the Southern Cameroons in November–December 1959, were dissected and two (6.9 per cent.) had infections of the *vivax* group.

G. haningtoni Newst. & Evans.

G. haningtoni is another rain-forest species which is particularly common in the Southern Cameroons.

A total of 59 specimens, collected between Mamfe and Victoria at the end of the wet season in 1959, was dissected and five (8.5 per cent.) had infections of the *vivax* group. No previous records of infections in *G. haningtoni* are known to the writer.

G. nigrofusca Newst.

In Nigeria and the Southern Cameroons this species inhabits rain-forest; elsewhere, as in Ghana (Nash, 1948), it will penetrate savannah. All the 182 specimens of *G. nigrofusca* referred to in Table I were collected at Ugbobigha; the results of 105 of the dissections have been taken from Page (1959c). Forty-four (24.2 per cent.) of the flies were infected. The proportion of *G. nigrofusca* infected at Ugbobigha was significantly greater than the proportion of *G. tabaniformis* and

G. fusca infected ($P < 0.001$ in both cases), although all three species had been collected from the same area of rain-forest. Jordan, Lee-Jones & Weitz (1961) showed that, whereas *G. tabaniformis* and *G. fusca* were feeding largely on red river hog, *G. nigrofusca* derived most of its food from Bovidae, especially bushbuck. Bovidae are important carriers of trypanosomes, and this may account for the greater proportion of infected examples of *G. nigrofusca* although, alternatively, as referred to above when discussing *G. tabaniformis*, some species may become infected more readily than others.

G. nashi Potts.

This species occurs in rain-forest in the Southern Cameroons, where it appears to be associated with *G. haningtoni*. Very few specimens have been caught and no data on infection rates are available.

The economic importance of the tsetse species in the area studied.

The infection rate in tsetse species probably depends both on the infectibility of the species and on whether or not the most widely used hosts are good trypanosome reservoirs. Ashcroft (1959) collected records on the incidence of trypanosomiasis in game in eastern Africa and concluded that the hosts on which the tsetse feed may be of considerable importance in determining the infection rate of tsetse flies and the relative proportions of the different species of trypanosomes. Although the data collected by Ashcroft are scanty, they suggest that there is considerable variation in the importance of different animals as trypanosome reservoirs and that this is not always directly related to the importance of the species as hosts for tsetse flies. The incidence of trypanosomiasis in some animals may be negligible, about 10 per cent. in wart-hog (*Phacochoerus aethiopicus*) and bush-pig (*Potamochoerus koiropotamus*), and 30–50 per cent. in others, including bushbuck, waterbuck (*Kobus* spp.) and other species of Bovidae. Ashcroft, Burt & Fairbairn (1959) found that various Bovidae (including the bushbuck) could be experimentally infected with *T. rhodesiense* and *T. brucei* and that the blood remained positive for a long period, whereas the species of Suidae investigated were usually infectible, but trypanosomes were very scanty in the blood. No comparable work has been carried out in West Africa but, assuming that the red river hog comes into the same category as the wart-hog and bush-pig, and that Bovidae are as heavily infected and as effective carriers of trypanosomes as in eastern Africa, data from southern Nigeria and the Cameroons provide further evidence for the conclusion that the identities of the main hosts of tsetse species are of considerable importance in determining the infection rates in the various species. The infection rate data presented for southern Nigeria and the Cameroons can be related to data on the natural hosts of each tsetse species in the same areas (Jordan, Lee-Jones & Weitz, 1961). Trypanosomes pathogenic to cattle were most frequently found in those species of tsetse—particularly *G. longipalpis* and *G. nigrofusca*—which obtained most of their food from Bovidae. *G. fusca* and *G. medicorum* also showed high infection rates and similarly derived most of their food from Bovidae, except in rain-forest at Ugbobigha where *G. fusca* took 73 per cent. of meals from red river hog, and only 21 per cent. from Bovidae, and where its infection rate was appreciably lower. *G. palpalis*, *G. tabaniformis* and *G. haningtoni*, which fed only to a limited extent on Bovidae, were much less heavily infected.

Some basic ecological information on certain of the tsetse species of the forest belt of Nigeria and the Southern Cameroons is available (Page, 1959*a*, *b* & *c*; Page & McDonald, 1959; Jordan, Lee-Jones & Weitz, 1961; Jordan, data on the ecology of the *fusca* group, as yet unpublished). By using this knowledge in conjunction with the data on trypanosome infection rates, some indication can be

reached of the economic importance of the different species of *Glossina* in these areas.

The comparative absence of Gambian sleeping sickness from the forest belt and coastal areas of Nigeria and the Cameroons is well established, but there are a few endemic foci in both belts (McLetchie, 1948). The rarity of human trypanosomiasis is not due to the absence of *Glossina*. *G. palpalis*, the main vector further north, is widespread, and *G. tachinoides*, another known vector, extends to the coast in the Eastern Region of Nigeria and was at least partially responsible for an outbreak of sleeping sickness at Eket (Macfie & Gallagher, 1914). *G. caliginea* also feeds readily on man, and this species may have been responsible for outbreaks of sleeping sickness in the creeks of the Niger delta—for example the 1934 outbreak in the Ahoada-Degema area of Owerri Province. Other species of *Glossina* in southern Nigeria are reluctant to bite man (Jordan, Lee-Jones & Weitz, 1961) and have not been implicated in the transmission of human trypanosomiasis. In northern Nigeria, evidence has suggested that optimum conditions for a high incidence of sleeping sickness are produced when there is close contact between individual tsetse flies and man (e.g., Nash, 1944, 1960). In southern Nigeria and the Cameroons, intimate contact between man and fly is generally rare, as the activities of neither are so restricted to the waterside as they are further north; the rarity of human trypanosomiasis in such areas is attributed largely to this impersonal man/fly contact (Page & McDonald, 1959). With such conditions prevailing, a low infection rate in the flies is to be expected; no gland infections have been recorded in *G. palpalis* from southern Nigeria or the Cameroons although, to the knowledge of the author, no dissections have been carried out where epidemics have occurred.*

Although human trypanosomiasis is rare in southern Nigeria and the Cameroons, animal trypanosomiasis is widespread and of great importance to the economy of the area. In northern Nigeria, humped Zebu cattle are kept, largely by the nomadic Fulani, and in the rains these are grazed in areas away from the fly-belts of *G. morsitans submorsitans* Newst. In the dry season there is more contact between cattle and fly, the Fulani preferring the risk of their beasts contracting trypanosomiasis further south to their death from thirst or starvation in the arid areas north of the fly-belts. In southern Nigeria, however, Zebu are not kept as they are too susceptible to trypanosomiasis; the only cattle normally owned by the local people belong to indigenous dwarf breeds, such as the Muturu, which have a greater tolerance to trypanosomiasis, but are slow-growing and very poor milkers. Larger breeds, such as the N'dama, which also have a considerable degree of tolerance, and even some cattle of Zebu type, are being introduced by Government departments to areas which support only limited fly populations. In areas of low trypanosome challenge, N'dama cattle do well under the local conditions, and their range could be greatly extended if it were not for the tsetse fly. The grazing in many areas of high challenge is probably good; for instance, three Zebu bait cattle kept at Ugbobigha, in the grasslands of northern Benin Province, with no supplementary feed, thrived when protected by two-monthly Antrycide Pro-salt injections.

G. palpalis is not considered to be an important vector of bovine trypanosomiasis in southern Nigeria because usually both the fly density and infection rates are very low. In many areas where cattle are kept by Government departments, *G. palpalis* is present in small numbers and the animals appear to be subjected to

* In June 1961, after this paper had been despatched for publication, the writer and Mr. D. A. T. Baldry of W.A.I.T.R. examined the salivary glands of 157 specimens of *G. palpalis* during a tsetse survey of part of Ogoja Division of Ogoja Province in the forest belt of the Eastern Region of Nigeria, where sleeping sickness is endemic, and found one fly with a salivary gland infection. In addition, 26 specimens of *G. palpalis* out of a total of 197 (13.2 per cent.) were infected with trypanosomes of the *vivax* or the *congolense* group. This represents a much higher infection rate than has been found in this species elsewhere in the forest belt (see Table II).

a low but continuous challenge, which is believed to stimulate an immune response. To prevent a change in environmental conditions upsetting the host/trypanosome relationship, it is advocated in some quarters that, when stock are distributed to other areas, they should be afforded temporary protection by receiving a single injection of a prophylactic drug. If such precautions are taken, it seems probable that cattle-keeping in areas of southern Nigeria where *G. palpalis* is the only tsetse species present could be greatly extended.

The importance of *G. tachinoides* as a vector of veterinary trypanosomiasis in southern Nigeria is not known; Nash (1948) quoted an instance when this species may have been responsible for bovine infections and for epizootics of *T. simiae* in pigs at Umuahia in Owerri Province.

G. caliginea is not considered likely to be of any importance as a vector of veterinary trypanosomiasis, as it is limited to areas generally unsuitable for cattle.

G. longipalpis frequents savannah grasslands, bites cattle readily and is heavily infected; it is considered to be of major economic importance, as it undoubtedly prevents the keeping of cattle in areas of potentially good grazing.

G. medicorum and *G. fusca*, both of which are relatively heavily infected, could be of economic importance if cattle were introduced into savannah grazing grounds where there was forest-island and riverine-forest vegetation.

The true rain-forest species, *G. pallicera*, *G. tabaniformis*, *G. haningtoni*, *G. nashi* and, in some areas, *G. nigrofusca*, are probably of little importance, however high their infection rate, since they do not appear to leave their normal habitat and thus do not penetrate potential cattle-grazing grounds. However, cattle trekked from the north to the markets of the south along routes passing through rain-forest must frequently contract trypanosomiasis from the forest-dwelling tsetse; but, as these animals are destined soon to be slaughtered, it is doubtful whether infections incurred so late on the journey are of great economic importance, although some loss of weight may occur before slaughter. Infections acquired in this way are probably particularly common in the forest belt of the Cameroons, where the human population is much sparser than in southern Nigeria, and the land much less extensively cultivated. Much forest still remains in the Cameroons and most roads pass through dense rain-forest. Tsetse flies belonging to *G. palli-cera* and the *fusca* group are numerous in this forest and have been observed feeding on cattle being trekked along the roads; one of the main cattle routes from the fly-free Bamenda grasslands (the Bamenda-Mamfe-Kumba and Mamfe-Ikom roads) passes through such country.

Summary.

Previous records and new data on infection rates in species of *Glossina* inhabiting southern Nigeria and the Southern Cameroons are presented, and the probable economic importance of each species is assessed.

The trypanosome infection rates were highest in those species of tsetse which fed largely on Bovidae.

The rarity of human trypanosomiasis in southern Nigeria and the Southern Cameroons is attributed largely to impersonal man/fly contact; under such conditions a low proportion of infected flies is to be expected. *G. palpalis* (R.-D.) is not an important vector of sleeping sickness, as it is in northern Nigeria. The incidence of infection in *G. palpalis* was generally very low; cases of bovine trypanosomiasis do occur in areas where *G. palpalis* is the only tsetse species present, but it is not considered to be an important vector.

Little information is available on *G. tachinoides* Westw. and *G. caliginea* Aust. in southern Nigeria; both have probably been at least partly responsible for some of the rare outbreaks of sleeping sickness reported from the south. *G. tachinoides* may be of some significance as a vector of animal trypanosomiasis.

The infection rate in *G. longipalpis* Wied. at the West African Institute for

Trypanosomiasis Research Field Station at Ugbobigha was 21.5 per cent.; this species is believed to be of major economic importance because its presence must prevent the keeping of cattle in large areas of potential grazing.

At least 13 per cent. of examples of *G. medicorum* Aust., *G. fusca* (Wlk.) and *G. nigrofusca* Newst. were infected in the populations examined, and the first two species are considered to be of economic importance because they penetrate potential grazing areas.

G. pallicera Big., *G. tabaniformis* Westw. and *G. haningtoni* Newst. are confined to rain-forest and were found to be lightly infected; they are therefore not considered to be of economic importance, except possibly as a source of infection to cattle on trade routes.

No data are available for *G. nashi* Potts.

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EXPERIMENTS ON THE CONTROL OF FRIT FLY, *OSCINELLA FRIT* (L.), ON LATE-SOWN OATS WITH SEED DRESSINGS AND 'LATE' SPRAYS.

By T. J. LEGOWSKI and H. J. GOULD

National Agricultural Advisory Service, Brooklands Avenue, Cambridge.

(PLATE V.)

Since 1952, investigations have been done at Cambridge on the control of the spring generation of frit fly, *Oscinella frit* (L.), on oats. In that year, in a replicated plot trial referred to by Thomas (1958), oats drilled on 22nd April were sprayed with 0.05 or 0.1 per cent. γ BHC on 19th May, when the plants were in the two-shoot stage and when approximately 30 per cent. of shoots were already showing symptoms of frit damage. By 29th May, there were 23 and 19 per cent. damaged shoots on the treated and 44 per cent. on the untreated plots, respectively. By mid-June, the difference in the appearance of the plots became quite striking (Pl. V, fig. 1), and by harvest time the treated plots appeared normal while the controls were nearly a failure.

This early trial demonstrated that it might be possible to prevent further frit damage and to save the crop by spraying when plants were already attacked; and although in the following years we investigated other aspects of chemical control, in particular seed dressings, since 1956 our attention has been mainly focused on the therapeutic value of what we call 'late' sprays, *i.e.*, sprays applied when symptoms of attack are already apparent, as opposed to the prophylactic sprays of DDT applied just before or at the time of egg-laying. The early preventive sprays of DDT give a reasonably good control of the first-generation attack (Thomas, 1958). However, in common with other preventive methods of control, they must be applied as an insurance and their big drawback is the difficulty of predicting with certainty when and where the outbreaks will occur. The late sprays on the other hand, if effective, could be applied only when necessary, a distinct advantage with a pest of fluctuating incidence.

Experimental methods.

Variety, location and plot layout.

With the exception of the National Agricultural Advisory Service co-operative trials, which are referred to separately in the text, the trials were done on a four-acre plot of land (sandy loam) near Cambridge, growing a mixture of cereals, vegetables and fruit, surrounded by wide strips of grass and adjoining agricultural land. The oats, variety Star in 1954 and Sun II in other years, were sown by hand drill in rows spaced at 7-8 in. at approximately 3 bushels per acre, usually in April, which is late for this area, but this increased the likelihood of a frit-fly attack. The size of plots varied from 81 sq. ft. in 1956 to 375 sq. ft. in 1959; they were separated by 2-ft.-wide paths or guard strips. The plot layout was a randomised block with three replicates in 1956 and four or five replicates in other years.

Assessment of the results.

This was based on counts of healthy and attacked shoots in 10 sample lengths of row per plot. The sample unit was two adjacent 1-ft. lengths of row, and counts were made along the diagonal on each plot. In the seed-dressing experiments the counts were made once or twice 3-5 weeks after the beginning of egg-laying,

i.e., when the damage was near its peak; in the experiments with late sprays they were done at the time of or just before spraying and again 10–14 days later. The numbers of damaged shoots were expressed as the percentage of the total and transformed into angles for analysis. The accuracy of this method of damage assessment has been discussed by Strickland (*in Anon.*, 1959).

When yields were measured, the oats were cut by hand, unless stated otherwise in the text, and passed through a small thresher and cleaner, and the results were corrected to 15 per cent. moisture content. In 1957, damage occurred on the plots due to birds, and in 1958 and 1959 the plots were protected with nets.

The seed-dressing trials.

These were done in 1954, 1956, 1958 and 1959. All the seed dressings contained 1–1.5 per cent. organo-mercury fungicide and the same quantity of organo-mercury was applied to the controls. The details of the treatments and the results are shown in Table I.

TABLE I.

Seed-dressing trials: treatments, sowing dates and results.

Year	Treatments	Rate (oz./bushel)	Date of sowing	Date of shoot count	Shoot attack (%)	
					1st count	2nd count
1954	γ BHC 40%	3	29.iv	3.vi	3.9	
	Control				13.8	
1956	Parathion 20%	6	21.iv	22.v, 6.vi	8.2*	24.0*
	Parathion 20%	3			23.3	
	Control				34.4	70.8
	Parathion 20%	6	12.vi	7.vii	14.3*	
	γ BHC 40%	3			22.3*	
	Control				40.1	
1958	Heptachlor 70%	2	11.iv	27.v	15.1	
	† Rogor 50%	4			17.5	
	Dieldrin 60%	2			14.9	
	Parathion 20%	6			15.1	
	Control				20.5	
1959	Dieldrin 30%	2	14.iv	19.v, 4.vi	31.0	44.3*
	‡ WL 1650 30% (dry)	2			25.4	43.4*
	WL 1650 30% (wet)	2			27.5	41.9*
	Control				33.8	58.9

* Significantly different from controls at $P=0.05$.

† O,O-dimethyl S-methylcarbamoylmethyl phosphorodithioate.

‡ 1,3,4,5,6,7,8,8-octachloro-3a,4,7,7a-tetrahydro-4,7-methanophthalan (Telodrin).

Although the seed dressings usually reduced the attack, the effect was appreciable only in 1956 with parathion, and to a lesser degree with γ BHC. Even in that year, however, the results with parathion varied considerably between the plots, and in 1958 parathion together with other materials was disappointing. In 1959, significant effects were detected at the second and not at the first count, but they were much too small to be of economic interest.

A seed dressing of γ BHC was also found to be partly effective by Walker (1953) and by Fidler & Webley (1956) who at the same time obtained rather better results with dieldrin. The latter was also used in the N.A.A.S. co-operative trials in

1958 (Thomas) but the results were variable and on the whole disappointing. Some of the possible reasons for this were examined by Way (1959).

No phytotoxic effects on oats were observed in any of our experiments with γ BHC or parathion seed dressings on the plots or in seed boxes in the greenhouse. As a rule, however, plants from seed dressed with parathion were taller and greener than the controls.

The 'late' spraying trials.

These were done every year from 1956 to 1960. Up to 1959 the object was to compare the effect of a single late spray with two early DDT sprays, and in 1960 to find out whether a 5–10 days' delay in application of late sprays would influence their effectiveness. In 1956 and 1957, DDT (miscible liquid) was used at 0.1 and in 1958 and 1959 at 0.2 per cent. active ingredient. The late sprays included the following materials, all of them miscible liquids: parathion at 0.1 per cent. in 1956 and 0.05 per cent. in 1957–60; dieldrin at 0.05 per cent., γ BHC at 0.05 per cent., Rogor at 0.5 per cent. and DDT at 0.2 per cent. They were applied at 100–120 gal. per acre, but parathion was also tested at 40–50 gal. per acre in 1959. In all experiments, 0.03 per cent. of a wetter (Agral LN in 1956–57, and Manoxol in 1958–59) was added to the sprays.

Timing of spray applications.

Every year observations were made on the first appearance and numbers of adult frit flies on the plots by sweeping with a net. As a rule the captured females were examined to determine the stage of egg development, and this was supplemented by searching samples of 20 to 30 plants for eggs. As soon as oviposition had begun, the first spray of DDT was applied, followed by the second spray 7–13 days later. The first flies were almost invariably found at the end of April or during the first three days of May and the eggs three to eight days later.

The early symptoms of damage were usually noticed in the second week of May and the attack was conspicuous and often severe by 18th to 20th May. This would probably be the time when a grower would wish to apply a treatment, and in most of our trials the late sprays were applied at this time. The dates of sowing, of spraying and of the shoot counts in each year up to 1959 are shown in Table II.

TABLE II.

Late spraying trials 1956–59: dates of sowing, of spraying and of shoot counts.

Date of sowing	1956	1957	1958*		1959
	21.iv	12.iv	11.iv	29.iv	14.iv
Date of spraying					
Early DDT sprays ..	4.v 11.v	3.v 13.v	2.v 15.v 19.v	9.v 19.v	30.iv 8.v
Late sprays					
DDT	—	—	3.vi	—	—
Parathion	19.v	20.v	3.vi	4.vi	19.v
Dieldrin	—	20.v	3.vi	4.vi	—
Rogor	—	20.v	—	—	—
γ BHC	19.v	20.v	—	—	—
Date of shoot counts					
1st count	22.v	17.v	27.v	3.vi	19.v
2nd count	6.vi	11.vi	16.vi	16.vi	4.vi

* In 1958 there were two separate trials.

Results.

The results are given in Table III.

TABLE III.

Late spraying trials 1956-59: summary of results.

Treatment	Damaged shoot counts (%)									
	1956		1957		1958a		1958b		1959	
	1st count	2nd count	1st count	2nd count	1st count	2nd count	1st count	2nd count	1st count	2nd count
Control	33.4	70.8	31.5	41.0	21.4	30.9	26.4	37.7	30.2	37.3
DDT (early) ..	9.6*	18.7*	6.4 *	2.1*	1.4*	0.6*	0.5*	1.9*	9.2*	20.3*
Late sprays (applied after first count) DDT					19.0	25.2				
Parathion (H.V.)	28.7	11.3*	30.1	11.9*	29.2	20.9	20.5	23.3*	33.6	23.9*
Parathion (L.V.)									30.4	19.8*
Dieldrin ..			34.1	13.4*	21.1	18.1*	19.6	24.1*		
Rogor			33.5	10.2*						
γ BHC	32.4	23.7*	27.1	10.6*						

Yields (cwt./acre)

Control	—	1.2	—	10.8	11.4
DDT (early) ..	—	2.4*	—	18.7*	16.9*
Late sprays Parathion (H.V.)	—	2.2*	—	18.1*	17.0*
Parathion (L.V.)	—	—	—	—	16.1*
Dieldrin ..	—	2.3*	—	14.0	—
Rogor	—	2.2*	—	—	—
γ BHC	—	1.8*	—	—	—

* Significantly different from controls at $P=0.05$.

The 1956 trial.—The results (Table III) show that after spraying there was a decrease in the percentage of damaged shoots on the γ BHC and parathion plots from the initial level of about 30 per cent., and an increase to about 70 per cent. on the control plots. Clearly, no further attack, or very little, took place on the treated and especially on the parathion plots after spraying, and they produced an acceptable stand while the controls were a failure. The possible reasons for the apparent decrease of damage on the sprayed plots will be discussed later.

The 1957 trial.—Four materials were compared: parathion, dieldrin, Rogor and γ BHC. At the time of spraying there were on the average just over two shoots per plant. The results were much the same as in the previous year: apparently all four materials were equally effective and again the figures suggest that no damage, or very little, occurred after spraying. Gamma BHC and early DDT gave better results than in 1956, possibly because the attack was less severe or not so prolonged.

Yields were too low, partly due to bird damage, to draw any conclusions. Nevertheless it was fairly obvious from the appearance of the plots that a single late spray could make the difference between a reasonable crop and a near or part failure.

The 1958 trials.—There were two separate trials. The first one drilled on 11th April could not be carried to the harvest stage due to gaps in the rows caused by faulty drilling. In this trial, three early DDT sprays were applied instead of the usual two due to heavy rain which fell almost immediately after the second spraying. As there was comparatively little damage on 27th May, and in order to obtain a heavier attack, the late sprays were not applied until 3rd June. By then, plants were more than 9 in. high and well tillered and past the stage most suitable for assessing the damage. The results were less striking although they followed the trend so obvious in 1956 and 1957: no increase but a decrease of damage after spraying with parathion or dieldrin. The late spray of DDT probably had little effect.

In the second trial, sown on 29th April in an attempt to obtain a heavy initial attack, the late sprays were again delayed till 3rd June. By then the plants already had on the average 3.9 shoots each and were approximately 6 in. high. By the time of the second count, on 16th June, the plants were about 9 in. high, well tillered and severely attacked by mildew, which made the counts more difficult and possibly less reliable than usual. The results show that the late sprays were less effective than previously; instead of the usual decrease of damage after spraying there was a slight increase on the sprayed plots, although significantly less than the increase on the controls. There was, however, a visible difference in the appearance of the sprayed and the unsprayed plots.

The yields, which were rather low, possibly due to late drilling and mildew, showed a distinct improvement on the late-sprayed plots, especially those treated with parathion, compared with the controls. Although the increase in yield on the dieldrin plots was not significant, four of the five replicates had yields much higher than the controls, but on one plot the yield was exceptionally low.

The 1958 microplot trial.—In 1958, in addition to the two main trials, there was a smaller one to obtain information on the effect of late sprays on larvae. This trial also served as a striking demonstration of the effectiveness of late sprays under certain conditions. The oats were sown on 15th May and each plot was ten rows wide and eight feet long. The first shoot count, on 6th June, made on ten 2-ft. lengths of row over the trial area showed that approximately 74 per cent. of shoots were damaged and there were then on the average 1.5 shoots per plant. The plots were sprayed on the same day, and there were three treatments: 0.05 per cent. parathion, 0.05 per cent. dieldrin and controls.

A difference in the appearance of the sprayed and unsprayed plots could be seen by 16th June. The second shoot count, made on 19th June, on three 1-ft. lengths of row per plot showed that there were on the average 6.7, 10.1 and 65.6 per cent. damaged shoots on the parathion, dieldrin and control plots, respectively. This was reflected in the appearance of the plots; in spite of the very heavy initial attack, the treated plots looked normal, while the controls were becoming a total loss (Pl. V, fig. 2).

The 1959 trials.—Parathion and dieldrin were applied on 19th May when just over 30 per cent. of shoots were visibly damaged and when on the average there were 3.3 shoots per plant. The results again showed a decrease of damage after spraying and an appreciable increase in yield over the controls. As in 1956, two early sprays of DDT gave no better control than a single late spray of parathion and there was little difference in yields. However, in 1959, the first DDT spray was applied when the first frit fly was caught by sweeping and five days before any eggs were found; better results would possibly have been obtained if the first spray had been applied a few days later.

The 1960 trial.—The results in 1958 suggested that late sprays may be less effective if they are delayed too long and when tillering is well advanced. This was investigated in 1960. Oats were sown on 28th April and a single spray of 0.05 per cent. parathion was applied to different plots at three dates: on 20th May, when about 35 per cent. of shoots were visibly damaged, on 25th May and on 30th May. At the time of spraying, 20 plants from the untreated plots were examined for the number of shoots and leaves. The plots were not harvested, due to gaps in the rows, but on 9th August five 1-ft. lengths of row were lifted on each plot and note taken of the number of plants, the number of panicles per plant and the weights of grain.

Spraying at any of the three dates had reduced the shoot damage (Table IV) but the earlier two treatments were significantly better than the last one. The sample yields were higher on the treated than on the control plots by 45, 38 and 25 per cent. in the order of spraying, and the highest yield was on the borderline of significance ($P=0.05$). There were on the average 1.38 panicles per plant on the treated and 1.19 on the control plants, but the difference was not significant.

TABLE IV.

The 1960 trial: effect of delay of spraying on frit-fly damage.

Spraying date	Damaged shoots (%)				Shoots/ plant	Leaves/ plant
	At spraying		Approx. 10 days later			
	Treated	Untreated	Treated	Untreated	Untreated	Untreated
20/v ..	35.3	35.4	10.8*†	34.0	1.4	1.5
25/v ..	35.3	38.7	12.9*†	36.1	3.0	3.1
30/v ..	29.0	34.0	16.4*	37.1	3.4	4.4

* Significantly different from controls at $P = 0.05$.

† Significantly different from the corresponding value for 30th May at $P = 0.05$.

The N.A.A.S. co-operative trials 1958-59.—Following the results obtained earlier in Cambridge, the Advisory Entomologists' Conference of the N.A.A.S. agreed in 1958 and more specifically in 1959 to compare late sprays with other treatments against shoot attack by frit fly, which were being tested on plots on commercial oat crops.

In 1958, a single late spray of dieldrin was included on seven sites and, on one of them, of Rogor also. The plot at each site occupied 1/150th acre. Unfortunately the shoot attack was very light; there was no damage on one site, on four sites the peak shoot damage was below 14 per cent. and only on two sites was it over 25 per cent. Of the six sites where some attack occurred, the damage on the late-sprayed plots 10-14 days after spraying was lower than on

the controls, on average by approximately 50 per cent., but significantly ($P=0.05$) so on only two sites. DDT plots were better than the late-sprayed plots but likewise significantly so on only two sites, and the level of attack was approximately 26 per cent. of that on the controls. For various reasons the yields could be compared on only three sites and there were no significant differences between the DDT, late-sprayed and the control plots.

In 1959 the following treatments were compared on ten sites: (a) two early DDT sprays applied at the time of egg-laying and before damage occurred; (b) one late spray of dieldrin or parathion applied when damage became conspicuous; (c) no sprays.

At nine sites the variety used was Sun II, and at one site, Eagle; and the treatments were replicated four times. The plots were approximately 1/150th acre on three sites and 1/40th acre on the others. Dieldrin was applied at all sites and parathion at six of them. The counts of damaged shoots were made at or near the time of late-spray application and again 2-3 weeks later. Yields

TABLE V.

The 1959 N.A.A.S. co-operative trials: the treatments and the results.

Site and sowing date	Date of spraying		Damaged shoots (%) at 1st and 2nd count and yields (cwt./acre)							
	Early DDT	Late sprays	Early DDT		Parathion		Dieldrin		Control	
			%	cwt./a	%	cwt./a	%	cwt./a	%	cwt./a
Cambs. .. 23/iv ..	11/v 21/v	28/v	17.2 16.8*	18.2*	26.5 25.0*	18.3*	23.3 24.7*	16.0*	27.2 32.4	15.1
Leics.** .. 4/v ..	19/v 3/vi	3/vi	40.2 40.6*	3.3*	47.1 43.6*	3.2*	46.0 42.9*	3.3*	52.4 47.5	2.2
Denbigh .. 23/iv ..	12/v 27/v	3/vi	16.7 18.9*	25.2*	25.7 27.5	22.1	27.1 30.5	22.3	25.0 29.2	19.4
Kent .. 21/iv ..	16/v 21/v	21/v	17.7 15.2*	11.4*	20.6 19.8	11.8*	18.9 14.8*	10.6*	20.0 20.4	8.6
Mons. .. 14/iv ..	5/v 19/v	26/v	7.6 6.9*	16.5*	—	—	12.5 12.6*	14.9	14.2 18.4	14.0
Hants. .. 13/v ..	27/v 10/vi	18/vi	9.2 12.7*	—	—	—	21.4 18.7*	—	22.1 24.7	—
Northumb.** .. 30/iv ..	25/v 9/vi	12/vi	26.3 39.5*	10.4	31.1 41.7*	11.0	33.9 44.2*	7.0	32.1 52.2	2.0
Somerset .. 20/iv ..	6/v 22/v	26/v	10.8 12.8*	10.2	—	—	20.4 18.1*	10.8	20.5 24.7	10.5
Montg. .. 14/iv ..	11/v 25/v	25/v	9.1 19.8*	35.6*	—	—	14.0 22.0*	32.6	16.0 30.9	31.7
Yorks. .. 5/v ..	19/v 2/vi	9/vi	— 26.8*	19.8*	36.5 36.0*	16.0*	35.4 39.2	15.9*	— 44.1	12.3

* Significantly different from the controls at a $P = 0.05$.

** In Leicestershire the plots were severely damaged by sheep. In Northumberland the yields were obtained from only two replicates, and are omitted from the discussion of results.

of grain were obtained from eight sites, by harvesting with a combine at three of them and by hand at the others.

In the main the results (Table V) agreed with those from the earlier Cambridge plot trials. Compared with the controls, dieldrin reduced the severity of attack significantly on eight out of ten sites and parathion on four out of six sites but this effect was much less marked than in our trials at Cambridge. Plant-growth data obtained at seven sites showed that the oats were already well advanced when late sprays were applied and on the average there were 2.3 shoots and just over four leaves per plant. On most sites the percentage of damaged shoots on the control plots increased only slightly between the date of late spraying and the second shoot count, which in view of only a slight increase in the numbers of shoots during that time (average 0.5 shoots per plant) suggests that the attack was past its peak when late sprays were applied.

On several sites the control obtained with DDT was disappointing as it reduced the damage by less than 50 per cent. In at least one case DDT was applied after some damage had already occurred, and this may possibly be also true for some of the other sites.

The yield figures showed that at one site there was no difference between the DDT plots and the controls, while on the other seven sites the yield on DDT plots was significantly better, on the average by 3.8 cwt. or approximately 25 per cent. Of the late treatments, yields on parathion plots were better than those on the controls on all five sites and significantly so on four; the average gain was approximately 25 per cent. Dieldrin yields were also better on all sites, but significantly on only four out of eight and the average gain was 1.6 cwt. or approximately 11 per cent. The yields on DDT plots were significantly higher than on parathion plots on only one site (Yorkshire), but higher than dieldrin on three sites (Cambridgeshire, Montgomeryshire and Yorkshire).

The effect of late sprays on larvae.

In nearly all our experiments the percentage of damaged shoots on the late-treated plots fell after spraying. In the absence of a further attack this apparent decrease of damage could be explained by tillering which was still taking place at that time and by the loss of some of the damaged and withered shoots. However, since during the same time the damage increased on the control plots which were tillering at least as well as the treated plots, it is clear that late sprays must have prevented further attack and damage on the treated plots. This could be due to the effect of sprays on adults, eggs or larvae. The small size of plots would greatly reduce any influence due to mortality of flies and the most likely explanation is the effect on larvae and possibly on eggs or on egg-laying. Although fewer frit-fly eggs are laid on DDT-sprayed plants (Legowski & Gould, 1958) there is no information whether parathion or dieldrin act in a similar way or how they affect the unhatched eggs. We found, however, by dissecting five or ten plants from each plot (8 days after spraying in 1952, 10 to 15 days in 1956, 1958 and 1959 and 6 days in 1960) that a high proportion of the larvae in the sprayed plants were dead or moribund (Table VI). A similar effect with higher concentration of parathion has been reported by Walker (1953).

It may be worth noting that the systemic materials demeton-methyl and Dipterex were tested, together with parathion and BHC in the 1956 trial. Demeton-methyl (0.037%) was applied on 12th May when oviposition was taking place and Dipterex (0.1%) on 12th May and on other plots on 19th May when about 30 per cent. shoots were visibly damaged. Both materials reduced the infestation only very slightly.

We also found that usually there were more larvae in the unsprayed than in the sprayed plants (Table VI). During the plant examination some of the dead

larvae, especially the very young ones, may have been missed; also, there were slightly more shoots per plant on the unsprayed than on the sprayed plants. However the difference in the number of larvae per plant was often large enough to suggest that the sprays prevented many of them from infesting the shoots.

TABLE VI.

Late spraying trials: larvae and pupae per plant and larval mortality.

No. of larvae recovered	1952		1956		1958		1959		1960	
	361		151		181		142		56	
	No./plant	% dead	No./plant	% dead	No./plant	% dead	No./plant	% dead	No./plant	% dead
γ BHC 0.05% ..	2.6	45	1.1	33	—	—	—	—	—	—
0.2% ..	3.7	29	—	—	—	—	—	—	—	—
Parathion 0.05% ..	—	—	0.5	94*	0.5	86	2.4	53	1.5	87
Dieldrin 0.05% ..	—	—	—	—	0.6	—	—	—	—	—
Controls	5.7	4	4.5	0	3.4	1	4.8	0	1.3	0

* Parathion at 0.1 per cent.

Recovery of damaged shoots after spraying.

In some of the experiments, notably on the microplots in 1958 there was a substantial reduction in number of damaged shoots after spraying. Thus in 1958 there were on the average eight damaged shoots per foot of row at the time of spraying and about two damaged shoots on the treated and 13 on the control plots 13 days after spraying. Some damaged shoots are likely to have wilted and disappeared but, even allowing for this and errors due to sampling, this difference seemed so large and the improvement in the appearance of the sprayed plots so striking that a possibility of recovery of shoots showing symptoms of attack had to be considered. Fidler (1958) also noticed a decrease in number of damaged shoots in some of his experiments following spraying with dieldrin and concluded that the damaged shoots must have recovered.

In 1959, we tried to obtain some information on the extent of such recovery by labelling and counting apparently healthy and visibly damaged shoots on ten plants on parathion and control plots 3 and 13 days after spraying. In this experiment (Table VII) a few damaged shoots disappeared, presumably by falling

TABLE VII.

Healthy and damaged shoots on ten plants 3 and 13 days after spraying with parathion (oats sown on 14th April).

	Sprayed (19.vi)		Unsprayed	
	22.v	2.vi	22.v	2.vi
No. shoots	44	56	44	76
No. damaged shoots	20	21	22	41
Percentage of damaged shoots ..	45.4	37.5	50.0	53.9
No. damaged shoots 'recovered' ..	—	1	—	0
No. damaged shoots disappeared ..	—	1	—	3

off, and only one out of the 42 initially damaged shoots could be said to have 'recovered'. Late spraying protected healthy shoots present and apparently most of those which formed later.

Damage to the young oat plant is characterised by the effect on the shoot which is composed of folded and unfolded leaves around and above the growing point. In 1959, we made observations on the stage at which larvae damaged the growing point by dissecting ten plants showing typical frit-fly symptoms at three dates in May. The first examination was made on 12th May, twelve days after the first frit fly was caught on the plots and seven days before the late sprays were applied. The plants had 2-3 shoots and the central shoot in each was showing damage. The second examination was made on 19th May when late sprays were applied to some plots and the third on 22nd May. The results were as follows:

Date	No. plants	No. shoots	Damaged shoots	Shoots with growing point damaged	Larval instar
12.v ..	10	25	11	1	II
19.v ..	10	33	10	7	II-III
22.v ..	10	53	22	18	II-III

It can be seen that little damage to the growing point occurred in the early phase of the attack; and it may be that, if larvae are killed at that stage, new leaves can be produced by the growing region to give the appearance of shoot recovery. This could be the explanation of the 'recovery' in 1958 and possibly in some of the other trials, though further more detailed work is desirable on this point.

The effect of loss of shoot on yield.

In spite of the initial loss of shoots, many of them primary ones, the yields on the late-sprayed plots were only slightly lower than on the DDT treated and much higher than on the control plots. A possible explanation is that an early loss of one shoot, even if it is a primary shoot, has little effect on yield and that serious loss occurs only after the destruction of the secondary shoots following the loss of the primaries; Cunliffe (1925) has shown this to be so in at least one of the two varieties with which he experimented. Late sprays probably prevent this later phase of the attack in addition to protecting the undamaged primary shoots.

In 1959, we tried to carry out a rather similar experiment to that of Cunliffe using the variety Sun II. On 14th April, 22 rows were sown by hand each with 20 seeds $\frac{3}{4}$ in. deep and at $1\frac{1}{2}$ - $1\frac{3}{4}$ in. spacing, the distance between the rows being $6\frac{1}{2}$ in. To reduce any possible effect due to size, the seeds were sieved and only 'main' grains used for sowing. To prevent frit attack, plants were sprayed with DDT on 30th April and 8th May and with parathion on 19th May.

On 12th May, plants were mainly in the three-shoot stage and frit damage was apparent on the adjoining unsprayed plots. An attempt was then made to kill the growing point of the leading shoots on all plants in every alternate row by piercing the shoot near the first node and in the region of the growing point with a fine needle. By 21st May it was clear that this treatment was only partly successful: on some plants the growing point escaped the injury while on others the lateral buds were also injured. In spite of spraying, some frit attack was taking place and there were some misses in the rows due to bad germination. It was obvious that the number of plants available at the end for comparison would be very much smaller than intended but, rather than discard the experiment, detailed records were made on 21st May and 2nd June of all plants with only the leading shoot

killed and of those showing damage to the leading and the secondary shoots. These plants, except those adjoining a gap in the row, were cut on 17th August and, for each, records were made of the number of panicles and of the weight of seed in each. The control plants were taken from the adjoining untreated rows and opposite the plants which were damaged. Results were obtained for 168 plants and these are given in Table VIII according to whether only the main shoot or more than one shoot (average 2.5) were lost.

TABLE VIII.

The effect of loss of shoots on yield.

	Controls	Leading shoot lost	Av. 2.5 shoots lost (incl. leading shoot)
No. plants	84	37	47
No. panicles/plant	3.24 \pm 0.15	3.26 \pm 0.28	3.30 \pm 0.29
Weight of seed/panicle (g.) ..	1.30 \pm 0.076	1.14 \pm 0.086	1.12 \pm 0.56
Weight of seed/plant (g.) ..	4.36 \pm 0.25	3.88 \pm 0.31	3.41 \pm 0.26

The data from this one experiment are insufficient to draw conclusions, and the results may vary with different conditions, especially with different seed spacing. However, together with Cunliffe's results they suggest that at least with some varieties the loss of the primary shoot alone may not necessarily have much effect on yield. Supporting evidence has been obtained during the dissection of healthy and damaged shoots on 19th May in 1959, referred to earlier. By then the development stage of the growing point in the secondary shoots which had replaced the damaged primary ones was already either the same or only slightly behind that of the healthy primary shoots.

Discussion.

These trials have shown that it is possible to obtain a worthwhile control of the first-generation frit-fly attack on late-sown oats by spraying at the time when symptoms of attack are already apparent. Providing the treatment is carried out as soon as damage becomes obvious, even a heavy attack can be brought under control. Of the materials tested, parathion was the best and dieldrin was probably nearly as good. Gamma BHC was also effective but in 1956 appeared to be inferior to parathion. Rogor was tested in only one trial and has shown promise.

Although, after spraying, the treated plots had less damage than the controls, the response to the treatments varied. It was best on the microplots in 1958, followed by the 1956 and 1957 and by the first of the 1960 trials; it was worst in the two trials in 1958. This appeared to be correlated with the growth stage at the time of spraying as indicated by the number of shoots per plant and also with the length of time the plants were exposed to attack before spraying. The younger the plant and the shorter the exposure, the better was the response to the treatment. To be most effective the late sprays should be applied within about 15 days of the beginning of egg-laying, by which time the damage should be obvious, and while there are no more than 1.5-2.5 shoots per plant. These conditions are most likely to be met with on late-sown crops which are particularly susceptible to serious frit-fly attack.

While the loss of grain on the controls compared with the early DDT plots was as much as 50 per cent., the yields on the late-treated plots, and especially on those sprayed with parathion, were generally only slightly lower than those on the DDT plots. However, the comparison with the early DDT sprays may not be

wholly relevant; field trials have shown (Empson, 1958) that, even on late-sown crops, routine DDT spraying need not necessarily be profitable, and the preventive application of two or three sprays of DDT is unlikely to become a commercial practice until reliable methods of forecasting the severity of attack are devised.

The effectiveness of late sprays probably depends on several factors. Killing of larvae hatching soon after spraying and of those already in the shoots; protection of the primary and secondary shoots still healthy, and the ability of the latter to make up for the early loss of the primary shoots; the recovery of attacked shoots and possibly even a growth stimulant action of the insecticides (Walker, 1953) may all have played a part.

The fact that late spraying can save a crop from serious damage or from total failure, as in the 1956 and 1958 trials, is obvious enough and would by itself be a sufficient argument for recommending the sprays in many cases. A gain of about 2.5 cwt. per acre would pay for the cost of spraying with parathion and a gain of about 1.6 cwt. per acre for spraying with dieldrin. The best available estimate of the likely loss of yield at a given level of shoot attack is that obtained from the N.A.A.S. experiments (Strickland, 1958). These have shown that on the average a loss of 2.5 cwt. per acre is likely to occur with about 10 per cent. shoots damaged at or near the peak of attack and excluding the subsequent loss due to grain attack. Allowing for errors and for a slight loss of yield that is probably unavoidable with late spraying and assuming that the yield response on commercial crops would be of the same order as in the N.A.A.S. trials and in our own, a figure of approximately 20 per cent. visibly damaged shoots 2-3 weeks after the beginning of egg-laying can be tentatively suggested as the minimum level at which the application of a late spray could usually be expected to pay for itself. In practice any advice on whether to spray would most probably be restricted to late-sown crops in years of serious attacks. Little difficulty should then arise in arriving at a decision which in addition to the extent of frit-fly damage should also take into account the date of sowing, the plants' stage of growth, the date of local frit-fly emergence and, if necessary, the density of unhatched eggs present on the plants.

Summary.

In plot trials with seed dressings on late-sown oats against shoot attack by frit fly, *Oscinella frit* (L.), chiefly near Cambridge, England, γ BHC, parathion, dieldrin, heptachlor, Rogor and WL 1650 (Telodrin) (1,3,4,5,6,7,8,8-octachloro-3a,4,7,7a-tetrahydro-4,7-methanophthalan) (dry and wet) reduced the attack but the effect was variable and for the most part not appreciable.

In 1952 and from 1956 to 1960, trials were made on the effect of late sprays of various insecticides applied to late-sown oats when symptoms of frit-fly attack were already conspicuous and sometimes severe. An advantage of this type of treatment over preventive sprays is that it ensures that the treatment is applied only where and when necessary. Single sprays of parathion, dieldrin, γ BHC and Rogor prevented or markedly reduced further attack and they were most effective when applied within about 15 days of the beginning of egg-laying and when plants were in the young seedling stage. Compared with the best treatment of two early preventive sprays of DDT there was only a slight reduction in yield. Parathion and to lesser extent γ BHC killed many larvae inside the shoots and probably prevented others from entering the shoots.

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FIG. 1. Control of *Oscinella frit*, 1952. Contrast, in mid-June, between plots sprayed with 0.05 or 0.1 per cent. γ BHC on 19th May and untreated plots.



FIG. 2. Control of *O. frit* with late spray of parathion (background) and dieldrin (foreground) in the 1958 microplot trial; an untreated plot in centre.

A SIMPLE METHOD FOR BREEDING THE HOUSE-FLY, *MUSCA DOMESTICA VICINA* MACQUART, IN THE LABORATORY.

By K. THARUMARAJAH * and E. S. THEVASAGAYAM †

*Insect-borne Diseases Field Training Centre,
Kurunegala, Ceylon.*

The most common house-fly in Ceylon is *Musca domestica vicina* Macq. *Musca d. nebulo* F. and *Musca sorbens* Wied. are also found in small numbers in houses. In nature, *Musca d. vicina* breeds mainly in decomposing refuse, cattle-dung heaps, human faeces and other decomposing organic matter.

Various substances have been used to breed the house-fly in the laboratory for experimental purposes. *Musca d. domestica* L. has been bred successfully in the laboratory by Hutchison (1916), Glaser (1924) and Grady (1928) using horse manure. Hockenyos (1931) used a mixture of horse manure and pig manure. Hafez (1948) describes a simple method where he used cotton-wool soaked in milk.

Large numbers of *Musca d. vicina* were required daily in the laboratory for testing insecticides. The breeding techniques mentioned above that had proved successful with *Musca d. domestica* were tried with *Musca d. vicina* without success. Feldman-Muhsam (1944) bred *Musca d. vicina* in cattle dung. Although, locally, this species breeds profusely in cattle dung in heaps, it could not be bred in this medium in the laboratory. It is observed that, in the field, this species does not generally breed in scattered cattle droppings.

Other substances, both natural and artificial, including wet bread, poultry manure and coconut poonac were tried. Of these, coconut poonac proved to be an excellent medium. Coconut poonac is the cake left over after the extraction of oil from coconut pulp and is sold as cattle food. It is very cheap and is easily available. Its percentage composition is as follows: oil 7-7.5, crude protein 21-22, carbohydrates 42-44, crude fibre 11-12, ash 5-6 and moisture 3-6 per cent.

Breeding method.

Moistened coconut poonac in a petri dish 4 inches in diameter is left in the fly holding cage for a day. The flies lay their eggs on the poonac and these hatch in 24 hours. The maggots are allowed to remain in this vessel for another day, after which the entire contents of the petri dish is transferred to a battery jar about 4 inches in diameter and 5 inches high. Powdered poonac is added to the jar to a depth of about 1 inch and the entire contents moistened. After another two days, a further quantity of powdered poonac is added to a depth of about 4 inches and the contents again moistened. The maggots live in the lower 3 inches of the jar. In another two to three days' time—about five to six days from hatching—the maggots are ready to pupate. By this time, the top layer to a depth of about 1 inch is quite dry although the lower regions, where the maggots are active, are very moist. The maggots now migrate to the top, dry layer and pupate there. The vessel may now be placed in the holding cage and the adults start emerging in two to three days, without any further attention. About 200-300 flies can be reared in a vessel of the type described.

The method described above is simple and requires no handling at all except

* Now attached to the Anti-Filariasis Campaign, Dehiwela, Ceylon.

† Now Entomologist at the Dry Zone Research Institute, Maha Illupallama, Ceylon.

for the first transference from the petri dish to the battery jar. This stage could not be avoided as the flies would not readily lay their eggs in poonac placed in the battery jar itself. Not only is coconut poonac readily available, but it can be stored indefinitely and converted into a breeding medium, when required, by moistening. It is not messy and does not produce any disagreeable odours, and all the developmental period is spent in the same medium, which makes this an excellent substance for breeding *Musca d. vicina* in large numbers in the laboratory.

Summary.

Coconut poonac, the cake left over after the extraction of oil from coconut pulp, has been used very successfully for breeding *Musca d. vicina* Macq. in the laboratory in Ceylon. The fly lays its eggs in the poonac and the larvae live and pupate in the same medium.

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LENGTH OF LIFE, FECUNDITY AND THE OVIPOSITION CYCLE IN
ANTHRENUS VERBASCI (L.) (COL., DERMESTIDAE)
 AS AFFECTED BY ADULT DIET.

By G. M. BLAKE, Ph.D.

*Agricultural Research Council, Pest Infestation Laboratory,
 Slough, Bucks.*

(PLATE VI.)

In southern England, adults of the varied carpet beetle, *Anthrenus verbasci* (L.), may be found in the open, feeding on the pollen and nectar of certain flowers from early May to the end of July. Males and females occur on flower-heads in approximately equal numbers and mating pairs may often be observed. The adults lay most eggs in dry birds'-nest material in the attics of buildings (Woodroffe, 1953) but some are laid on woollen materials and other animal products. The behaviour reactions which direct adults to flowers and back to suitable oviposition sites are not completely understood. However, adults have been shown to be attracted by the odours of both hogweed flowers (*Heracleum sphondylium*, Umbelliferae) and birds'-nest material (Blake, unpublished) and it is assumed that, in nature, olfactory responses assist in guiding females to both flowers and oviposition sites. Collections of adults have been made from 21 genera of plants belonging to 7 families. Nearly all the flowers were small and creamy-white or pale pink and most belonged to the Umbelliferae, Rosaceae and Compositae. The two plants most attractive were hogweed and the cultivated spiraeas (*Spiraea* spp., Rosaceae). Under outdoor temperature conditions in southern England the incubation period of the eggs is about seven weeks but the larvae require at least one and usually two years in which to complete development (Blake, 1958, 1959). In the spring the fully grown larvae pupate within the last larval skin and the newly formed adults remain inactive (still within the larval skin) for a further short period before finally emerging as active beetles.

There is very little detailed knowledge of the length of life and fecundity in *A. verbasci*. The complexity of the adult behaviour, involving visits to flowers concluding or perhaps alternating with visits to oviposition sites, necessitated, if the life of the adult were to be understood, detailed knowledge of the fecundity and oviposition cycle and the effects upon them of adult nutrition.

Methods.

Fully grown larvae were collected from cultures (see Blake, 1958) and kept in petri dishes at 20°C. and 70 per cent. R.H. until pupation. Examination of pupae (0-1 day old) revealed a difference between the sexes; the female possesses two clearly visible papillae on the posterior end of the abdomen which are absent in the male (Pl. VI). With transmitted light, these papillae can be seen through the surrounding ecdysed larval skin, without disturbance of the pupa.

Male and female pupae were kept individually at 20°C. and 70 per cent. R.H. and observed daily. The duration of the inactive adult stage of each individual was noted. On emergence (0-1 day old) from the old larval and pupal skins the active adults were weighed, paired and placed in 2 in. x 1 in. glass tubes. The preferred oviposition substrate has been shown to be woollen flock (sterilised

woollen sweepings from a mill floor) (Blake, unpublished). A small quantity of this flock, well teased out and made as even in texture as possible by removing small pieces of string and yarn, was added to each tube.

Diet.

The natural diet of the adults is, so far as is known, restricted to pollen, nectar and possibly rain-water or dew. The experimental diet was kept as near as possible to the natural diet.

It was not possible to collect pollen in sufficient quantity from hogweed or *Spiraea* spp. but pollen from the michaelmas daisy (*Aster* sp.) was obtained from the Wright Fleming Institute of Microbiology by courtesy of Dr. A. W. Frankland. It was considered that this pollen would be satisfactory, for, although beetles had not been collected from this plant in particular, Composites generally were attractive to the beetles. There does not appear to be any information on the composition of this pollen. Todd & Bretherick (1942) collected data from the literature on 13 complete pollen analyses and stated that, in general, there was a wide range in protein (7.9 to 40 per cent.), starch (0.8 to 11.1 per cent.), sugar, fat and ash, and that undetermined matter made up nearly 50 per cent. of the samples. A more reliable source of protein was achieved by supplying the beetles with flake egg albumen (Edward Gurr, Ltd.) ground with a pestle and mortar until the particle size was of the same order as that of the pollen when compared under a low-power microscope.

The 'nectar' was obtained by making up a solution of equal proportions of glucose, sucrose and fructose with a total solution strength of 18 g./100 ml. The sugars were of laboratory reagent quality and the solutions were made up with distilled water.

The equivalent of dew or rain-water was supplied to the beetles as distilled water.

The sugar solution and distilled water were given to the beetles by means of well soaked polyporus strips. The excess liquid was removed with filter paper, and the strip dusted with pollen or egg albumen and placed in the tube so that it adhered to the side of the glass. The food remained in the tube and was always available to the adults; the strip was renewed daily at the time of the egg count.

The above foods were combined to form six different diets, and in the control group the beetles were deprived of both food and water; the diets were sugar solution alone, sugar solution with pollen, sugar solution with albumen, water alone, water with pollen, and water with albumen.

Statistical methods.

Since the experiments involved more than two groups and more than one variable simultaneously, the groups were compared by the methods of analysis of variance and covariance described by Goulden (1939). Providing the variance ratio, F (Fisher, 1946) was significant, the means were compared by a 't' test of the form $(M_1 - M_2) \sqrt{(S.E._1^2 + S.E._2^2)}$ together with the table of 't' from Fisher (1946). Throughout the paper, differences between means or variances are said to be significant if the value of P is below 0.05.

Weights of adults at emergence.

An analysis of variance showed that there were no significant differences in mean emergence weight, i.e., weight on the first day of active adult life, between the seven experimental groups. This was true for males and for females. However, there was a sex difference; the females were significantly heavier than

the males in all groups except the control group (deprived of food and water) and the group given water with albumen (Table I). For correlation of emergence weight and sex with length of life and fecundity see page 467.

TABLE I.

Significance of the difference in mean weight on emergence (*i.e.*, on first day of active adult life) between males and females for each diet separately and for the combined data.

Adult diet	Males			Females			Significance of difference (<i>P</i>) in mean emergence weight between males and females
	No. of indivs.	Mean emergence weight (mg.)	Standard error	No. of indivs.	Mean emergence weight (mg.)	Standard error	
No food or water	22	2.864	0.173	22	3.159	0.192	NS >0.05
Water	25	2.844	0.152	25	3.644	0.187	S <0.01
Water with pollen	27	3.100	0.114	26	3.581	0.173	S <0.05
Water with albumen	28	2.861	0.138	28	3.232	0.148	NS >0.1
Sugar solution	21	2.550	0.114	22	3.152	0.118	S <0.01
Sugar solution with pollen	25	2.672	0.148	25	3.244	0.195	S <0.05
Sugar solution with albumen	22	2.777	0.164	22	3.332	0.210	S <0.05
Combined data	170	2.821	0.055	170	3.342	0.071	S <0.001

Length of life.

Duration of inactive adult life (unmated).

The presence or absence of a sex difference in the length of the inactive adult stage was investigated under one set of conditions, 20°C. and 70 per cent. R.H. The results are shown in Table II. The difference between the means was so small compared with the daily recording interval that no statistical comparison could be made.

TABLE II.

Comparison of the lengths of the inactive adult life of males and females at 20°C. and 70% R.H.

	Length of inactive adult life (days)								Number of indivs.	Mean length of inactive adult life (days)
	4	5	6	7	8	9	10	11		
Frequency for males	2	2	6	46	88	21	3	0	168	7.7
Frequency for females	0	2	12	83	56	9	5	1	168	7.5

The effect of temperature and humidity on the period of inactivity was determined at 10, 15, 20 and 25°C. with 30, 70 and 90 per cent. R.H. at each temperature. The period decreased with rise in temperature and at the two higher temperatures it tended to be a little shorter at 90 per cent. than at 70 per cent. or 30 per cent. R.H. (Table III).

TABLE III.

Effect of temperature and relative humidity on the length of the inactive adult life.

Temp. (°C.)	R.H. (%)	No. of indivs.	Mean length of inactive adult life (days)	Range (days)
10.0	30	—	—	—
	70	7	32.4	7—47
	90	8	31.0	7—44
15.0	30	18	16.8	4—24
	70	19	17.0	13—23
	90	19	17.2	12—21
20.0	30	18	8.1	4—12
	70	18	8.1	5—11
	90	20	6.6	3—11
25.0	30	6	3.7	1— 8
	70	7	3.8	3— 5
	90	7	2.3	1— 4

Earlier workers have also made observations on the length of this inactive stage or ' quiescent adult life ' ; their findings are given in Table IV.

Duration of active adult life (mated).

There were no significant differences between the lengths of life of beetles deprived of food and water and those given water, water with pollen and water

TABLE IV.

Data from the literature on the length of the inactive adult life.

Author	Experimental conditions	Length of inactive adult life (days)	
Kalandadze (1927) ..	Room temp. (Germany)	Ranged from 4—7	
Kunike (1939)	16—18 °C. 20—22 °C. Dish in the sun	Ranged from 7—8 Ranged from 5—6 Ranged from 4—5	
Griswold (1941) ..	Room temp. (New York)	Males	Females
		5.74 ± 0.36 Range 1—8	5.15 ± 0.29 Range 3—8
Kuwana (1950) ..	Room temp. (20 °C.)	Ranged from 2—7	
Kiritani (1958b) ..	20 °C.	4.89	
	25 °C.	4.68	
	30 °C.	3.25	

with albumen. This was true for both males and females and there were no differences between the sexes. When sugar solution was given, there was a significant increase in length of life in both sexes, and when albumen was given in addition to sugar solution there was a further increase in both sexes. There was a sex difference in the effect of pollen when given with sugar solution—the males lived longer than when given sugar solution alone or sugar solution with albumen, but the females did not live significantly longer than when given sugar solution alone (Table V).

TABLE V.

Effect of diet on the length of life of the active adult at 20°C. and 70–90 per cent. R.H.

Adult diet	Males			Females		
	No. of indivs. (N)	Mean length of life (days)	S.E.	No. of indivs. (N)	Mean length of life (days)	S.E.
No food or water	22	18.2	0.8	22	22.1	1.3
Water	25	18.8	0.8	25	25.3	1.6
Water with albumen	28	19.8	0.8	28	26.0	2.4
Water with pollen	27	20.9	0.7	26	26.1	0.6
Sugar solution	21	41.7	3.1	22	54.8	2.3
Sugar solution with albumen	22	58.3	3.2	22	69.5	7.2
Sugar solution with pollen ..	25	67.0	2.7	25	60.6	3.3

A difference between means of less than 6.82 (N_1 and $N_2=28$) is not significant, whilst a difference greater than 7.96 ($N_1=21$ and $N_2=22$) is significant.

TABLE VI.

Data from the literature on the length of the active adult life.

Author	Experimental conditions	Adult diet	Length of active adult life (days)	
			Males	Females
Yokoyama (1929)	May & June (Japan)		30	40–50
Kunike (1939) ..	Room temp. (Berlin)	No food or water	13–14 (7–24)	13–17 (7–41)
Griswold (1941) ..	Room temp. (New York)	No food or water	12.74 \pm 0.74 (8–21)	16.76 \pm 1.21 (9–39)
Kuwana (1950) ..	Room temp. (20 °C.)		About a fortnight (5–30)	
Kiritani (1958a) ..	30 °C.	No food or water	8.52 \pm 1.75	10.19 \pm 1.24
		Water	12.50 \pm 3.51	13.50 \pm 3.51
		Honey	10.66 \pm 2.87	14.70 \pm 2.17

The figures in brackets indicate the range.

Comparison of the above figures with those of earlier workers is difficult because only Kiritani (1958a) experimented under controlled temperature conditions; the available information is tabulated below (Table VI).

Oviposition.

Preoviposition period.

The preoviposition period varied from 3 to 14 days (Table VII). There appeared to be a tendency for the onset of oviposition to be delayed in beetles which were given food compared with those deprived of food and water.

TABLE VII.

Frequency with which oviposition began on the 4th, 5th, 6th or a later day of active adult life.

Adult diet	No. of individuals	First day of oviposition									
		4	5	6	7	8	11	12	13	15	
No food or water	22	5	13	4	—	—	—	—	—	—	
Water	23	1	3	11	8	—	—	—	—	—	
Water with albumen	25	—	6	8	7	2	—	—	—	—	
Water with pollen	24	—	3	8	8	4	1	—	—	—	
Sugar solution	21	—	11	8	2	—	—	—	—	—	
Sugar solution with albumen ..	21	—	6	8	4	2	—	—	1	—	
Sugar solution with pollen ..	26	1	6	12	4	1	—	1	—	1	

Oviposition cycle.

When the oviposition rate (eggs per female per day) was plotted against time, three clearly defined peaks of oviposition occurred on about the sixth, twelfth

TABLE VIII.

Periodicity of peaks in the oviposition rate.

Adult diet	Age (days) at which peaks in the oviposition rate occurred			Ovipositing life (days)	Mean length of active female adult life (days)
No food or water	5	10	14	4th—24th	22.1
Water	6	12	16	4th—22nd	25.3
Water with albumen	6	13	17	5th—19th	26.0
Water with pollen	6	13	17—18	5th—23rd	26.1
Sugar solution	6	12	17	5th—48th	54.8
Sugar solution with albumen ..	6	12	17	5th—70th	69.5
Sugar solution with pollen ..	6	12	17	4th—58th	60.6

and seventeenth days (fig. 2). The age at which the peaks occurred varied slightly with the diet of the beetles, tending to be earlier in those deprived of food and water (Table VIII). Only those beetles given a diet containing sugar continued to lay after the end of the third peak and in these the rate of oviposition dropped considerably and the replicate pairs got out of step so that there were no further distinct peaks in the oviposition rate (fig. 1).

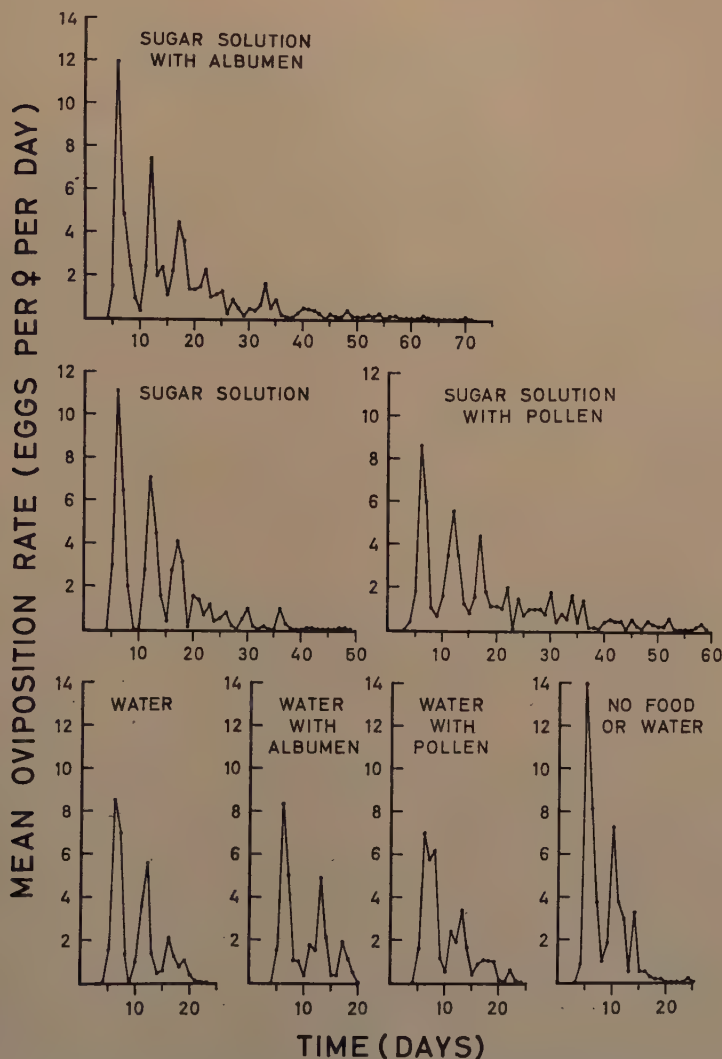


Fig. 1.—Mean daily oviposition rate in *A. verbasci* for beetles deprived of food and water and for beetles given six different diets.

Fecundity.

The mean total fecundity of beetles deprived of food and water was 50.3 eggs. It was somewhat surprising to find that when beetles were given water, water with pollen or water with albumen there was a significant decrease in the mean

egg output, which was reduced to 34.4, 34.5 and 32.9, respectively (Table IX). A diet of sugar solution increased the mean egg output to 58.8, *i.e.*, above the value for beetles deprived of food and water, but the increase was not significant until pollen or albumen was given in addition to sugar solution (Table IX). The three highest individual records for egg output occurred when the beetles were given sugar solution with pollen (120 and 115 eggs) and sugar solution with albumen (106 eggs).

TABLE IX.

Number of eggs laid in each of first three peaks, after third peak, and the mean total fecundity per female.

Diet	No. of individual pairs (N)	Mean no. of eggs laid per female				The mean total fecundity per female
		1st peak	2nd peak	3rd peak	after 3rd peak	
Water with albumen ..	28	17.6	10.9	3.9	0	32.9
Water with pollen	26	21.0	9.6	4.1	0.8	34.5
Water	25	17.5	11.8	6.4	0	34.4
No food or water	22	27.8	16.5	5.2	0.1	50.3
Sugar solution	21	23.3	16.2	10.3	8.3	58.8
Sugar solution with pollen ..	26	18.6	16.1	11.1	18.9	64.9
Sugar solution with albumen	22	21.9	15.0	12.3	18.7	68.1

A difference in mean total fecundities greater than 14.5 ($N_1=26$, $N_2=28$) is statistically significant but one less than 12.9 ($N_1=21$, $N_2=22$) is not. The mean total fecundity per female and the mean number in the first peak have been adjusted for variations in female weight, whilst the other figures have not, which accounts for the discrepancies in the totals.

Dick (1937) has grouped the oviposition cycles of Coleoptera into four distinct types:— (1) species which live only a short time as adults and lay all their eggs within a few days, (2) species of which the adults live a long time and produce eggs continuously, (3) species which during one season lay eggs in batches at fairly short intervals of time, (4) species which lay more or less continuously in two or more seasons separated by a period during which oviposition ceases. Dick (1937) gives *A. verbasci* as an example of a beetle with oviposition of the first type, quoting Yokoyama (1929) who states that 100 eggs are produced in from 1 to 2 weeks. There seems little doubt from the present work that in fact *A. verbasci* falls into group 3, for the majority (*i.e.*, over 70 per cent.) of the eggs were laid in three batches on approximately the sixth, twelfth and seventeenth days (Tables VIII and IX, and fig. 2). The remainder were laid irregularly throughout the female life. There is no mention in the literature of the eggs of *A. verbasci* being laid in batches. However, unless the ages of the beetles in the group being tested were the same, to within a day, peaks in the oviposition rate would not be distinct. Since few of the earlier writers have claimed this degree of accuracy, this may well explain the omission.

Earlier records of fecundity and oviposition taken from the literature are shown in Table X.

Correlation of emergence weight and sex with length of life and fecundity.

There was a significant correlation between weight on emergence (Table I) and the length of life of males when they were given water, water with pollen, or water with albumen, and of females when they were given water with pollen or water with albumen. There was no significant difference between the three regression coefficients for males or between the two for females; the mean regression coefficients were $b=3.04$ and $b=7.07$, respectively. There was a significant difference between the mean regression for males and for females.

There was a significant correlation between the weight of females on emergence and their fecundity when deprived of food and water ($b=16.65$), given water alone ($b=10.97$) and given water with pollen ($b=14.98$).

Discussion.

It is significant that *Anthrenus verbasci*, deprived of food and water and kept at between 70 and 90 per cent. relative humidity, lived sufficiently long (about three weeks) to lay 74 per cent. of the maximum recorded number of eggs, i.e., a mean total fecundity of 50.3 eggs compared with the maximum of 68.1 obtained when the beetles were given sugar solution with albumen. Nor did such deprivation reduce the viability of the eggs, for the percentage hatch of eggs laid by adults collected from cultures (i.e., deprived of food and water) was similar to that of adults collected from flowers (Blake, 1958).

Drinking water, whether given alone or with pollen or with albumen, did not lead to an increase in length of life and actually reduced the fecundity (this latter result is discussed later).

A. verbasci can assimilate sugars; the length of life was significantly increased when sugar solution (glucose, fructose and sucrose) was given (Table V). That sugar increases the length of life of adult insects has been demonstrated by several workers. Fraenkel (1936) found that blowflies, *Calliphora vicina* R.-D. (cited as *C. erythrocephala* (Mg.)), live only 2-3 days given water alone but given sugar and water live for 1-2 months. Glaser (1923) showed that *Musca domestica* L. lives longer given sugar and water than when deprived of food and water. Larson & Fisher (1924) showed that *Callosobruchus maculatus* (F.) (cited as *Bruchus quadrimaculatus* F.) and Norris (1934) showed that *Cadra cautella* (Wlk.) and *Anagasta kühniella* (Zell.) live longer given sugar and water than water alone.

Sugar did not significantly increase the fecundity above the level of those deprived of food and water (Table IX).

As well as being able to utilise sugar to maintain life, *Anthrenus verbasci* seems able to assimilate proteins for this purpose but only when they are given in conjunction with sugar (cf. protein with water). The length of life was increased by about 40 per cent. for males and about 27 per cent. for females, when they were given protein with sugar compared with sugar alone (Table V).

In most insects, with the exception of the Lepidoptera which lack proteolytic enzymes (Norris, 1934), proteins are essential for egg-production. In *A. verbasci* it is only when sugar and protein occur together in the diet that fecundity is significantly increased (Table IX). A similar result was obtained by Glaser (1923) for *M. domestica*. He showed that adult house-flies given protein lived only a short while and laid very few eggs, but on a diet of protein with sugar their length of life and fecundity reached the maximum.

As mentioned earlier, the figures for total fecundity per female for the various experimental groups showed that the fecundity of beetles given water, water with pollen, or water with albumen was less than the fecundity of beetles deprived of food and water (Table IX). It has not been possible to explain this result but detailed examination of the experimental procedure shows that all the groups were not treated in exactly the same way. The polyporus strips on which the liquid food was supplied were not present in the control group (no food or water). The

TABLE X.
Data from the literature on fecundity and oviposition.

Author	Experimental conditions	Diet	Preoviposition period (days)	Ovipositing life (days)	Rate of oviposition	Total fecundity per female	Range (eggs)
Yokoyama (1929)			15-16	7-14			20-100
Kunike (1939) ..		No food or water	4	3-4	Maximum on 2nd day	30	13-14
Kuwana (1950) ..	Room temp. (20 °C.)		2	14	50% by 5th day	50	-100
Kiritani (1958a)	30 °C. 30 °C. 30 °C.	No food or water Water Diluted honey	2.95 ± 0.48 2.00 + 1.24 1.57 + 0.54	2.90 ± 0.73 6.60 + 2.08 6.86 + 2.47		40.3 ± 6.81 72.20 + 17.25 60.57 + 15.90	

presence of the liquid raised the relative humidity, for a part of each day, to between 80 and 90 per cent., compared with the figure of 70 per cent. for the controls (no food or water). However, it is difficult to see how either of these variables could depress the fecundity by a significant amount.

Several features point to earlier maturation in beetles completely deprived of food and water compared with those given a diet:—

- (1) the preoviposition period was one day less (Table VII),
- (2) the peaks in the oviposition rate occurred earlier, *i.e.*, on the 5th, 10th and 14th days compared with the 6th, 12th and 17th days (Table VIII),
- (3) the number of eggs in the first and second peaks was greater: the numbers of eggs in the first peak for each experimental group were analysed statistically and it was shown that beetles deprived of food and water laid significantly more eggs than beetles given water, water with albumen and sugar solution with pollen (Table IX).

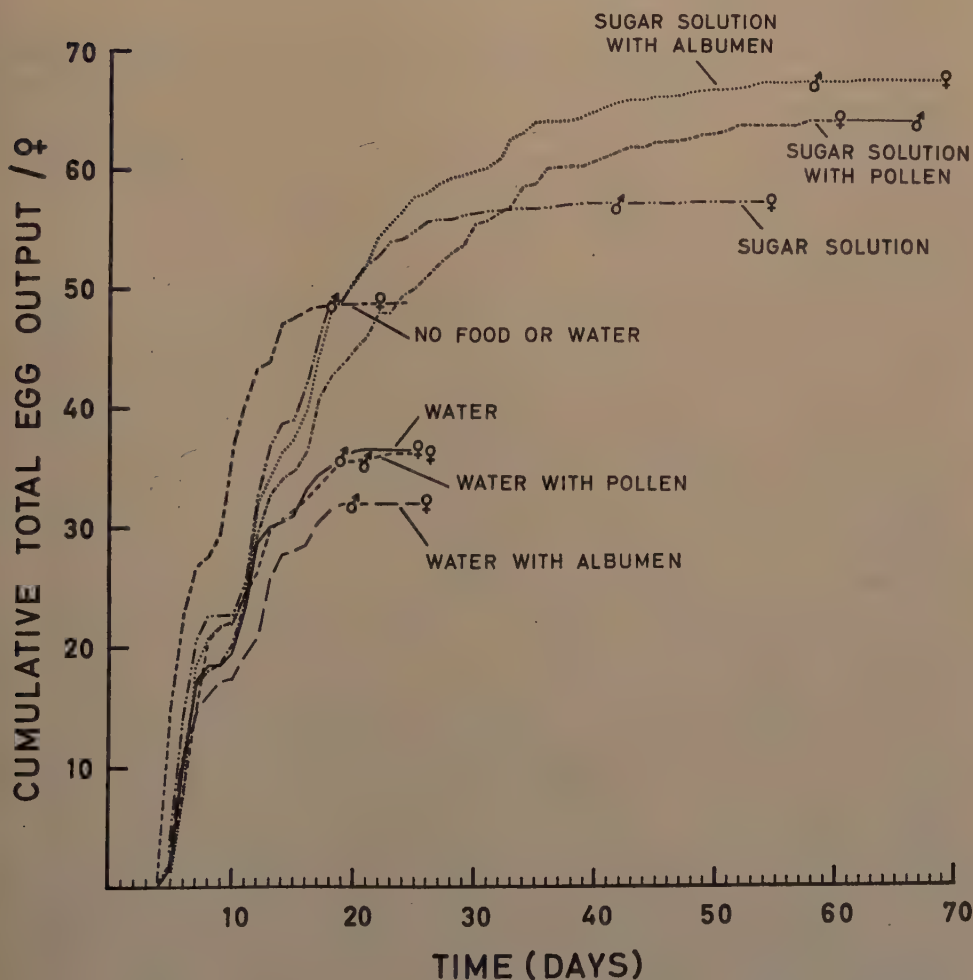


Fig. 2.—Cumulative total of the daily means of egg output in *A. verbasci* for beetles deprived of food and water and for beetles given six different diets. The mean length of life of males and females on each diet is indicated by the positions of the sex signs (♂, ♀).

The effects on total fecundity of an initially low rate of oviposition combined with a short adult life may be seen in fig. 2. This graph shows that the oviposition rate of beetles given water, water with pollen and water with albumen was lower than for beetles deprived of food and water and this combined with a similar length of life resulted in a lower total fecundity for the sugar-less diets. For the beetles given sugary diets, although the oviposition rate was initially lower it was maintained for longer. The beetles deprived of food and water did not maintain their oviposition beyond the third peak. Beetles given sugar lived much longer than those deprived of food and water, and continued to lay eggs for some considerable period after the end of the third peak (fig. 2). These later eggs raised the total fecundity of beetles given sugar solution with pollen, or sugar solution with albumen, to a significantly higher figure than that for the controls with no food or water. Larson & Fisher (1924) reported a similar tendency for *Callosobruchus maculatus*; they found that "food reduced the number of eggs laid in the first few days of oviposition, but lengthened the time over which eggs were laid."

The effect of pollen on length of life and fecundity was similar to the effect of albumen with one or two minor differences; pollen with sugar solution provided a better source of energy for males than for females—judging from the figures for length of life (Table V).

In conclusion it would seem that adults deprived of food and drink achieve only about a third of the maximum length of life but lay in that time about 75 per cent. of the maximum egg output. Sugar doubles the length of life and the addition of protein approximately trebles it. The fecundity is significantly increased when the diet includes both protein and sugar—an increase of about 25 per cent.

These facts suggest that populations can thrive and increase when adults are deprived of food and water. Visits to flowers, for pollen and nectar, increase length of life and fecundity so that they are of direct advantage to the species. Aggregation on flowers also provides indirect advantages; for instance, it increases the chances of mating, especially in areas of low population density. Moreover, flower visitation encourages dispersal of the population, since adults do not always, if at all, return to oviposit in the original larval habitat. This may well be the explanation for the rapid spread of *A. verbasci* in typical suburbs (Woodroffe & Southgate, 1954) where ribbon development has provided a string of suitable habitats.

Summary.

A study has been made of the length of life, fecundity, oviposition and the effects upon them of adult nutrition in the varied carpet beetle, *Anthrenus verbasci* (L.).

The length of the inactive (unmated) life for males and females at 20°C. and 70 per cent. R.H. was 7.5 and 7.7 days, respectively. This period, which is spent in the moulted last larval skin, decreased with rise in temperature, from a mean of 32.4 days at 10°C. to 3.8 days at 25°C. Humidity differences had relatively little effect.

The effects of various diets on the length of life, oviposition cycle and fecundity of active adults were observed. The experimental diets were water, water with pollen, water with albumen, sugar solution, sugar solution with pollen, sugar solution with albumen, and the control group in which the beetles were deprived of food and water. The sugar solution was a mixture of equal parts of glucose, sucrose and fructose in water.

There were no differences in length of life between male and female beetles (males 18.2–20.9 days, females 22.1–26.1 days) given water, water with pollen, water with albumen and those deprived of food and water. But there was an

increase for both sexes when the beetles were given sugar solution, and a further increase, to a mean of 58.3 days for males and 69.5 for females at 20°C. and 70–90 per cent. R.H., when albumen was added to the sugar solution. However, although sugar solution with pollen increases still further the length of life of the males, that of the females was no different from what it was on sugar solution alone.

The preoviposition period ranged from 3–14 days with a mode about the fourth day.

The oviposition cycle consisted of three clearly defined peaks of oviposition on about the 6th, 12th and 17th days. There was a similar pattern in all the groups except that those given sugar solution continued to lay, at a low rate, after the end of the third peak.

The fecundity of beetles deprived of food and water was 50.3 eggs; for beetles given water, water with albumen and water with pollen there was a significant decrease; for beetles given sugar solution there was an increase which became significant when pollen or albumen were given in addition to the sugar. Maximum fecundity occurred on a diet of sugar solution with albumen.

There was significant correlation between length of life and weight on emergence for males given water, water with pollen, or water with albumen, and for females given water with pollen or water with albumen. There was significant correlation between fecundity and emergence weight for females deprived of food and water, given water alone and water with pollen.

Acknowledgements.

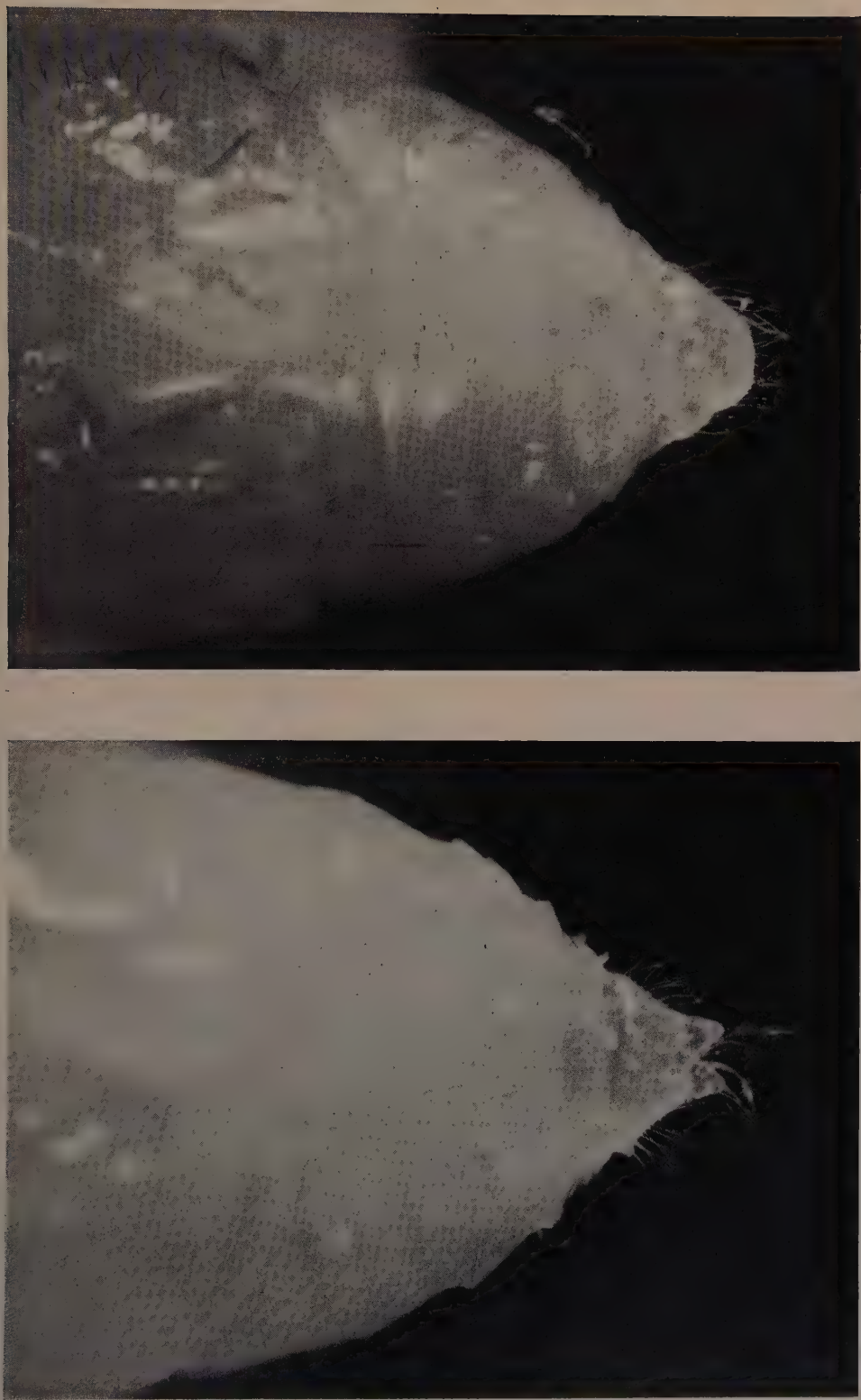
I am indebted to Mr. G. V. B. Herford, C.B.E., Director of the Pest Infestation Laboratory, for permission to present an account of this work to the University of London as part of a Ph.D. degree thesis, and to Mr. M. E. Solomon for much helpful discussion and criticism.

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Pupal abdomen of *Anthrenus verbasci*: female (left); male (right). The old larval skin normally surrounding the pupa has been removed in order to show more clearly the difference between the sexes.

PYGOSTOLUS FALCATUS (NEES) (HYMENOPTERA, BRACONIDAE),
A PARASITE OF *SITONA* SPECIES (COLEOPTERA, CURCULIONIDAE).*

By CONRAD LOAN

Entomology Research Institute for Biological Control,
Belleville, Ontario, Canada

and

F. G. HOLDAWAY

Department of Entomology and Economic Zoology, University of Minnesota,
St. Paul 1, Minnesota, U.S.A.

A programme to introduce insect parasites into Canada to aid in control of the sweetclover weevil, *Sitona cylindricollis* Fhs., was begun in 1952.† Three species of Braconids were released: *Microctonus aethiops* (Nees) auctt., *Perilitus rutilus* (Nees) and *Pygostolus falcatus* (Nees). The life-history and immature stages of the species were studied to provide a background for the parasite introduction. This paper is an account of the immature stages and biology of *P. falcatus*, a parasite obtained from Sweden by the Entomology Research Institute for Biological Control, Canada Department of Agriculture, Belleville, Ontario. Similar information on *M. aethiops* and *Perilitus rutilus*, together with the methods and results of the biological control attempts, will appear in succeeding papers.

Distribution and hosts.

Pygostolus falcatus is recorded in Great Britain from *S. lineatus* (L.) and *S. hispidulus* (F.) by Jackson (1922). In the U.S.S.R., Grossheim (1928) reared the species from *S. lineatus*, *S. crinitus* (Hbst.), *S. humeralis* Steph. and *S. inops* (Gylh.); Meyer (1934) from *S. crinitus*, *S. inops* and *S. humeralis*; and Ulashkevich (1935) from *S. lineatus* and *S. crinitus*. Díaz (1923) reared *P. falcatus* from the adult of the Curculionid, *Polydrusus pilosulus* Chev., in Spain. In the present study, *Pygostolus falcatus* was reared from *S. lineatus*, *S. hispidulus*, and *S. humeralis* collected in Sweden. It was released in Manitoba, Canada, in 1958, and the immature stages were recovered from *S. cylindricollis* collected in the release area in 1959 and 1960.

Methods.

Larvae of *P. falcatus* were reared at Belleville, Ontario, from *S. lineatus* and *S. hispidulus* collected near Kattarp, Sweden. Weevils to be used as hosts

* Contribution from the Entomology Research Institute for Biological Control, Research Branch, Canada Department of Agriculture, Belleville, Ontario, Canada, and Paper No. 4524 Scientific Journal Series, Minnesota Agricultural Experiment Station, St. Paul 1, Minnesota, U.S.A. From a thesis submitted by C. Loan to the Faculty of the Graduate School of the University of Minnesota in partial fulfilment of the requirements for the Ph.D. degree, 1960.

† A similar programme was begun in Minnesota in 1953 by the second author in co-operation with T. L. Aamodt, State Entomologist, Minnesota State Dept. of Agriculture and Dr. H. L. Parker of the U.S.D.A. European parasite laboratory in France. The first author worked on this project while holding a research assistantship on the project. The Minnesota programme was integrated with the Canadian programme when arrangements were made for the first author to pursue his research for the Ph.D. degree on the parasites of *Sitona* species. (F. G. H.)

were collected near Belleville and determined to be parasite-free by rearing, and by dissection of samples. Adults of *S. cylindricollis*, in groups of varying size, were exposed to female parasites for periods of from under one hour to more than 24 hours: usually ten weevils were placed with a single parasite or

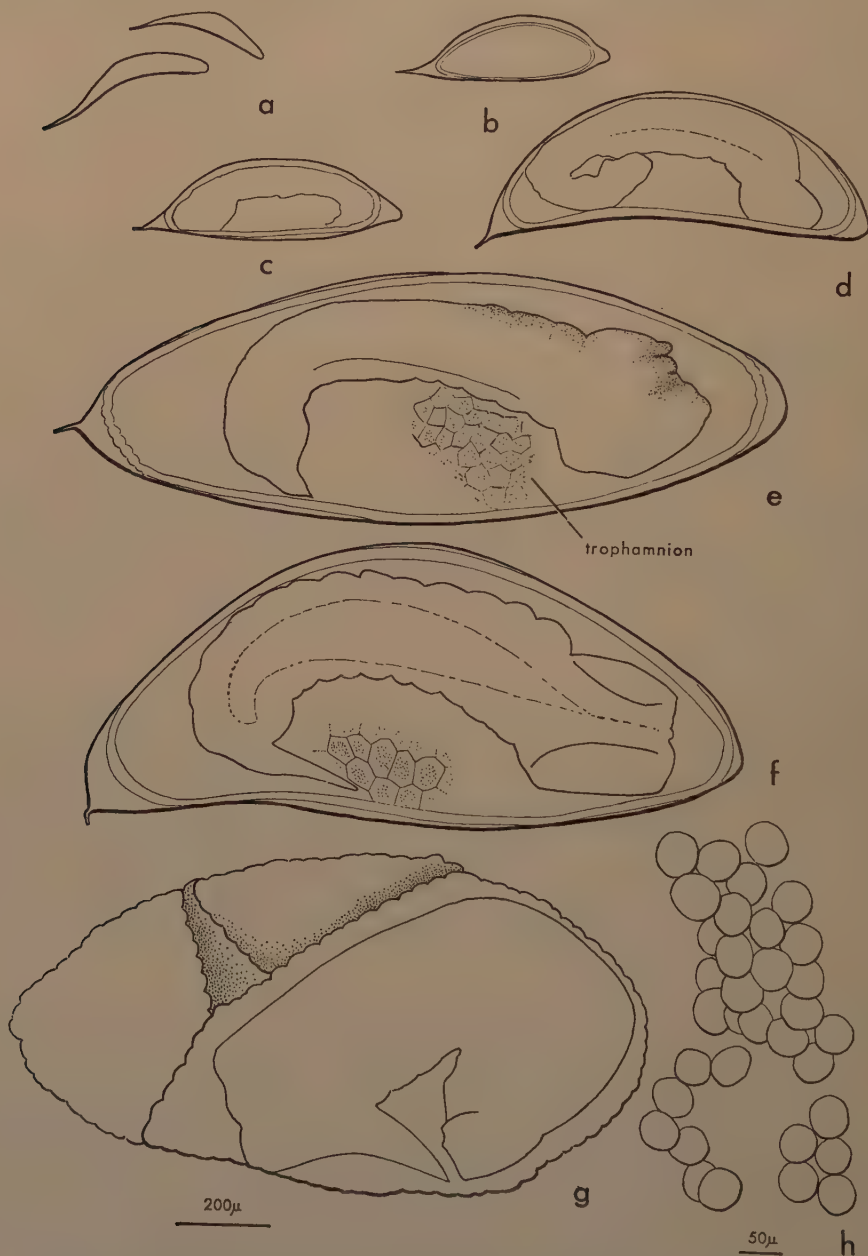


Fig. 1, a-h.—Development of the egg of *Pygostolus falcatus*. a, ovarian egg; b-f, eggs one to five days old, respectively; g, larva hatching; h, cells of trophamnion dissociating.

50 or more weevils with 25 parasites. Individuals were removed from holding cages at selected intervals and dissected in 0.8 per cent. saline solution to determine the degree of parasite development.

The immature stages were sketched with the aid of a camera lucida. First-instar larvae were anaesthetised by one or two drops of cocaine hydrochlorite or 3 per cent. chloral hydrate in the dissection fluid and larvae in more advanced instars were immobilised without distortion by immersion in warm water. The cuticle was examined for characters to separate the larval instars. The delicate transparent cuticle was separated from the body tissue by placing the larvae in 95 per cent. alcohol and was then stained in alcoholic fuchsin and mounted in diaphane for further study. The number of larval instars (five) was determined from the cast skins adhering to the apex of the abdomen of the penultimate-instar larva (fig. 3d-e). Four of the five larval instars are free in the haemocoel of the weevil. The final-instar larva is ensheathed within the cuticle of the fourth-instar larva until the moment of emergence from the host. Thus the discrete larval cuticles are not readily observed as the sclerotised cuticle of the developing fifth-instar larva conceals the transparent cuticle of the fourth-instar larva.

The adult parasites were maintained at 74°F. and a relative humidity of 45-55 per cent. in small wooden cages with a sliding lucite front and a plastic mesh back. Undiluted granulated honey, dotted on strips of stiff paper taped to the wall of the cage, served as food, and water was available from saturated dental cotton. The rate of oviposition and the fecundity were determined from individual females maintained in wooden cages (2×2×3 in.) similar in design to the larger cages described above. A female, on emergence, was provided with ten adults of *S. cylindricollis*, which were replaced with fresh ones at 24-hour intervals. The exposed weevils were dissected each day for recovery of the parasite eggs.

Descriptions.

Adult and ovaries.

The external morphology of the adult was discussed by Marshall (1889). The female is stramineous in colour when reared at 74°F., but those that emerged from cocoons held at 50-60°F., or in field cages in early autumn, were dark brown.

The ovaries are paired and each consists of three to seven ovarioles attached distally to one another. Each ovariole of the newly-emerged female contains two to four mature eggs and numerous immature ova. The rounded cephalic end of the egg is oriented away from the oviduct.

Females that died without ovipositing contained large numbers of eggs and immature ova. A female six days old contained 78 mature eggs; one ten days old, 88 mature eggs; and one 21 days old, 96 mature eggs. Females with access to weevils contained fewer eggs: a female 11 days old which had deposited 46 eggs contained 21 mature eggs and immature ova; a female ten days old which had deposited 28 eggs contained 20 mature eggs and immature ova.

Egg.

Length of ovarian egg 307-338 μ (314) * including pedicel, width 72-81 μ (77), length of pedicel 95-113 μ (104). The egg is concave-convex, with the pedicel narrow and slightly twisted right of centre. The egg increases in volume after deposition. Its form in relation to age after oviposition is shown in fig. 1. The chorion is transparent, thin and smooth, and stretches to accommodate the growth of the embryo and trophamnion. Some mature eggs were enclosed only by the trophamnion, a circumstance indicating that the chorion had been torn away by the expansion of the egg.

* Minimum and maximum measurements of ten specimens of the immature stages are given, followed by the mean in parentheses.



Fig. 2, a-h.—First to third larval instars of *Pygostolus falcatus*. a, first-instar larva six days and 21 hours old; b, anterior view of first-instar head capsule; c, lateral view of head capsule of developing first-instar larva; d, lateral view of head capsule of diapause first-instar larva; e, apex of tail of first-instar larva; f, falcate mandible of first-instar larva, and mandible of aberrant first-instar larva; g, second-instar larva five days and 23 hours old; h, third-instar larva nine days and 21 hours old.

The embryo lies freely within the space enclosed by the trophamnion. It is immersed in clear fluid and may drift slightly from one end of the egg to the other. Its body is either curved, with the tail touching the abdomen, or straight. The mature embryo is visibly segmented and the head capsule is distinct and lightly sclerotised.

Larva.

The nomenclature of Short (1952) is followed in the descriptions of the larvae.

First-instar larva.—Larva (fig. 2a) caudate in form and characterised by a dark grey head capsule, a slender, 13-segmented body, and a sharp, prominent tail. Length of newly-hatched larva, 1125μ (one specimen); diapause larva, $1462\text{--}1620\mu$ (1521), mature larva, $2115\text{--}2409\mu$ (2292), caudal appendage (tail) $248\text{--}315\mu$ (275), head capsule $189\text{--}230\mu$ (216), width $203\text{--}261\mu$ (243). Head in anterior view (fig. 2b) with falcate mandibles, prominent, deeply sclerotised external rim of the hypopharynx, and inconspicuous, non-sclerotised antennae; in lateral view, ventral lobe of head of mature first-instar larva (fig. 2c) greater in depth than that of the diapause larva (fig. 2d). Abdominal cuticle sculptured by a pattern of hexagonal and pentagonal cells ($\times 100$ and not cleared with KOH): tail (fig. 2e) with scale-like setae 6μ long.

An aberrant form of the first-instar larva was found occasionally with the head capsule incompletely sclerotised and the mandibles spoon-shaped rather than falcate (fig. 2f).

Second-instar larva.—Similar in general form to the first-instar larva (fig. 3g), all structures of the head and abdomen non-sclerotised. Length $1743\text{--}1829\mu$ (1786) (two specimens). Head transparent and without a head capsule, with four lobes and a distinct oral aperture in anterior view; mandibles broadly pointed, flat, apex 14μ long and 12μ wide, total length about 30 microns. Width between tips of mandibles 63μ , mandibles inserted five microns beneath dorsal lobe; cuticle smooth; one distinct, stout, seta 5μ long on each side of oral aperture and several setae within the aperture leading to the mouth. Abdomen linear, similar to first instar, not grub-like, creamy white in colour; tail short, smooth, within the cast tail of first instar; spiracles absent.

Third-instar larva.—Larva (fig. 2h) grub-like, with lateral ampullae and white urate cells beneath cuticle of abdomen, cuticle non-sclerotised. Length $2282\text{--}2988\mu$ (2490).

The following characters of the head were evident with fuchsin stain: labial sclerite weak, closed dorsally and ventrally; a thin structure dorsad of the labial sclerite to the base of each mandible; apex of mandible $12\text{--}18\mu$ long (up to 36μ long if basal area included), mandibles $60\text{--}102\mu$ (82) apart, flat, situated on either side of the anterior rim of the hypopharynx, dorsal and anterior to the developing mandibles of fourth instar.

Abdominal segment X rounded, insertion of tail ventral and somewhat anterior; tail 45μ long, smooth, enclosed by cast skins of second and first instars; spiracles absent.

Fourth-instar larva.—The fourth-instar larva (fig. 3) is readily distinguished by a characteristic chaetotaxy, weak development of the sclerites of the head, and sclerotised mandibles. Length $2822\text{--}4150\mu$ (3469), whitish-yellow in colour, grub-like.

Head in lateral view either extended forward or retracted into prothorax; in anterior view labial sclerite incompletely closed dorsally and open ventrally;

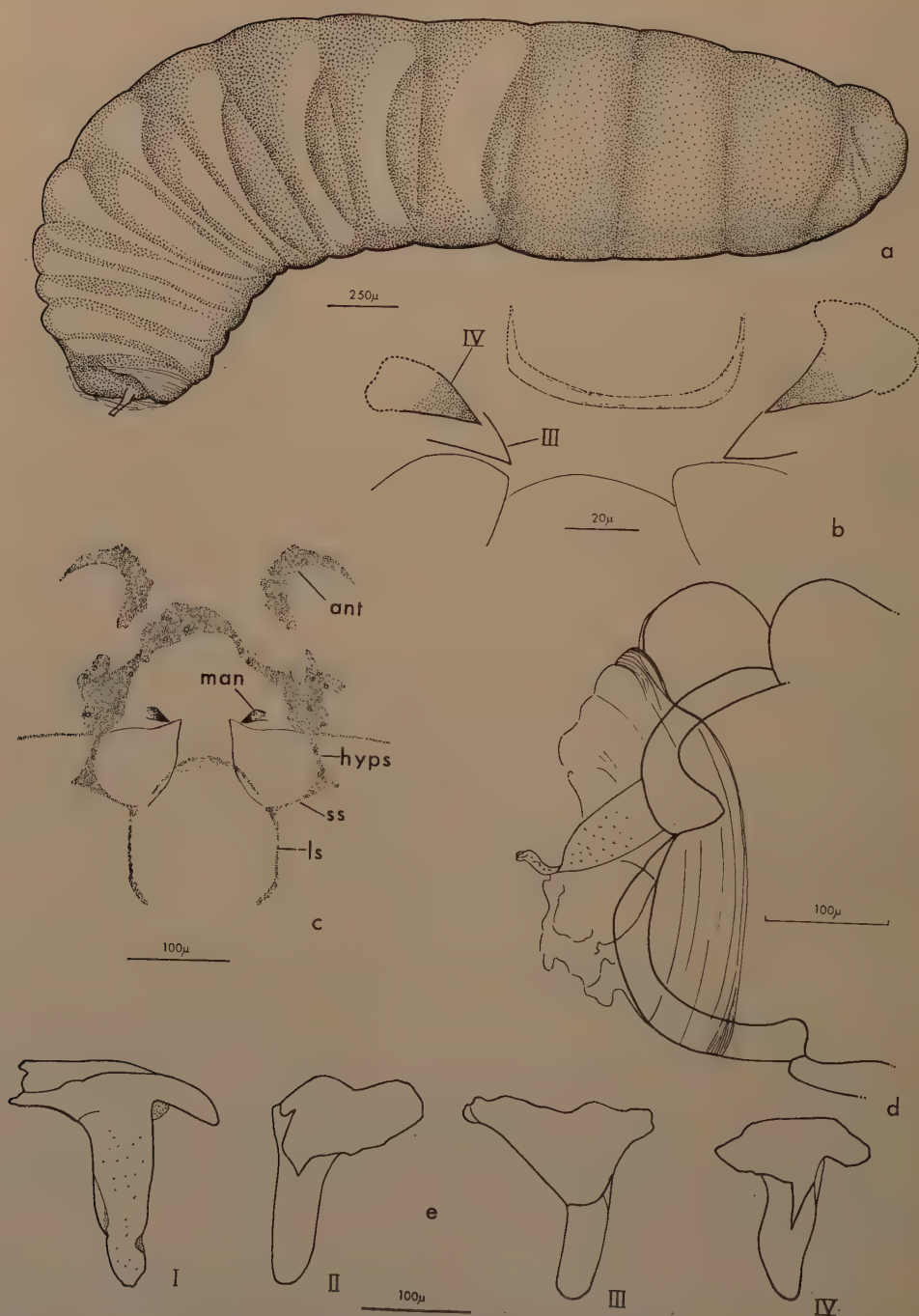


Fig. 3. a-e.—Fourth larval instar of *Pygostolus falcatus*. a, dorso-lateral view of larva 10 days and 22 hours old; b, mandibles of fourth instar beneath non-sclerotised mandibles of third instar; c, anterior view of non-stained head showing antenna (ant), mandible (man) and hypostomal spur (hyps); d, apex of abdomen showing complex of cast skins; e, cast larval tails and excised tail of fourth instar of fig. 3d separated, numerals refer to the instar to which each tail belongs.

stipital sclerite incomplete; an uneven, lightly sclerotised band across labroclypeal region with sensillae, united to stipital sclerite by hypostomal spur; mandibles simple, slightly curved and sharp, apex $18-24\mu$ (22) (including basal portion $54-66\mu$ (58)), distance between mandibles $64-84\mu$ (73), antennae oval, flat, distinctly outlined; irregular patches of microsetae.

Abdominal segments with folds and creases at the ampullae; cast skins of previous instars, including the first, retained at tip of abdomen; prominent white urate cells in fat-body; cuticle wrinkled by rounded protuberances; microsetae on thorax and abdominal segments I to V sclerotised, brush-like, on the head, sparsely, in patches. Closed spiracles on mesothorax and abdominal segments I to IX (evident only with fuchsin stain).

Fifth-instar larva.—Length $3320-4150\mu$ (3860), grub-like, dull yellow in colour, thorax and anterior abdomen pale grey, head marked by striking areas of sclerotisation (fig. 4). Head with labial and stipital sclerites, hypostoma, pleurostoma deeply sclerotised: hypostoma incomplete, attached internally to the tentorial bar; stipital setae (fig. 4) about 7μ long; mandibles simple, similar to those of fourth instar, 90μ apart, apex 30μ long; maxillae distinct, lightly sclerotised dorsally, maxillary palps slightly elevated, each with area of sclerotisation and one or several sensillae, setae 12μ long; labial palps similar to maxillary palps; post-labial setae up to 15μ long, scattered, labroclypeal area lightly sclerotised, lateral and dorsal borders slightly infolded, numerous spherical sensillae, scattered setae 7μ long; antennae oval, flat, clear, dorsal and lateral borders sclerotised.

Cuticle of abdomen, thorax, and head roughened by rounded to sharp protuberances 6μ in height and width and with scattered setae 6μ long; spiracles on mesothorax and abdominal segments I to VIII, 23μ in diameter; tail small and spike-like.

The fifth-instar larva of *P. falcatus* is similar to the larva of *P. sticticus* (F.) as described by Short (1952, pp. 76-79).

Prepupa, pupa and cocoon.

The abdomen and thorax of the prepupa (fig. 4d) are better differentiated than in the final-instar larva. The developing compound eyes are prominent in the prothorax. The pupa is of the exarate Hymenopterous type. The cocoon is compact, smooth, greyish-white in colour and torpedo-shaped. The anterior end is more tapered than the posterior, and the compound eyes of the developing adult become visible as development proceeds. The cast of the final-instar larva appears within the cocoon as a characteristic black patch on the inner, dorsal, posterior surface.

Biology.

Parasitism, fecundity and length of life.

Like other members of the BLACINAE, *P. falcatus* is endoparasitic. Reproduction is parthenogenetic and thelytokous. No males were reared from European weevils in this study and none appeared in four generations propagated in the laboratory, though according to Jackson (1928), the male of *P. falcatus* has been found. Stich (1929) reared males and females of a species that he cited as *P. multiarticulatus* (Ratz.) from the weevil, *Otiorhynchus laevigatus* (F.). According to Allen (1948), *P. multiarticulatus* is a synonym of *P. sticticus* (F.), but some other systematists regard the two as distinct species. A single egg is deposited by *P. falcatus* in the haemocoel of an adult of *Sitona*. The female does not discriminate between parasitised and non-parasitised weevils, and as many as 15 eggs and first-instar larvae were recovered from a single host. Recognition of the host weevil was indicated by the alert stance of the parasite and its rapid walk to one

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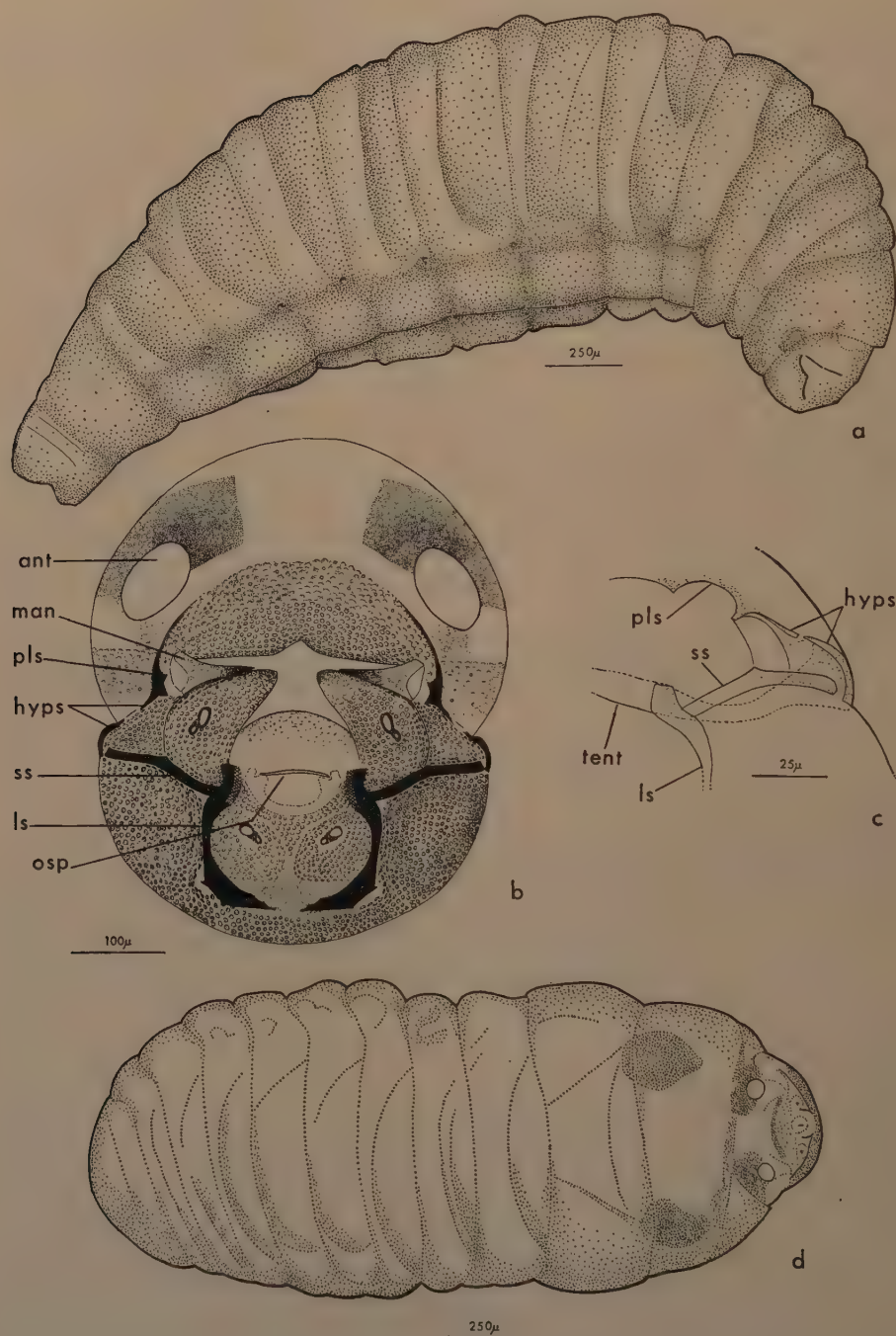


Fig. 4, a-d.—Fifth larval instar and prepupa of *Pygostolus falcatus*. a, lateral view of larva emerged from host weevil; b, anterior view of head showing pleurostoma (pls), hypostoma (hyp) and orifice of silk press (osp); c, sclerites in relation to the tentorial bar (tent); d, prepupa.

side of the weevil before oviposition. The weevils ignored the parasites and did not attempt to escape or move away from them, and often the parasite avoided weevils in a cage by stepping or flying to one side.

To oviposit, the female leaps on to the elytra of the weevil and immediately inserts its ovipositor into the apex of the abdomen. The oviposition thrust is not always successful: in 12 observed attempts on five weevils only two parasite eggs were recovered. Oviposition may be attempted several times while the parasite remains astride the weevil. Repeated thrusts may be made within a few seconds of each other or some minutes after the initial thrust. Often the parasite remains on the back of the host weevil for as long as 20 minutes, and on one occasion a parasite was observed to remain on a host for 50 minutes. In such instances other parasites may be attracted to the weevil until two or three parasites are one on top of the other. The weevil may fall from the cage wall or foliage when attacked, but this does not always dislodge the parasite.

Parasitism occurred during both the day and night. One female maintained in complete darkness at 74°F. for eight days parasitised 25 weevils out of 80 exposed. The daily parasitism of eight females in field cages was determined from the time of their emergence. The number of weevils parasitised per female in a 24-hour period varied from zero to eight; the minimum and maximum mean numbers parasitised per female were 1.1 and 3.8. The maximum number of weevils parasitised in an 11-day period was 39, or 35.4 per cent. of the 110 weevils exposed to the parasite.

The species readily parasitised *S. scissifrons* Say, *S. flavescens* (Marshall), *S. cylindricollis* and *S. japonicus* Roel. A preference for one or more of these species was not apparent in the laboratory. The legume weevils, *Hypera meleus* (F.) and *H. nigrirostris* (F.), were rarely parasitised in the laboratory although one example of *H. meleus* was found with five parasite eggs.

The daily oviposition of eight females in field cages was determined also from the time of their emergence. The number of eggs deposited per female varied from 0 to 11; among these females the average daily oviposition varied from 1.2 to 5.0 eggs and the maximum number of eggs laid by an individual female was 46.

Females of *P. falcatus* at 74°F. lived 5 to 15 days (mean of 11.0 days for 20 parasites); in field cages, in June 1956, they lived 7 to 11 days (mean of 9.4 days for eight parasites); in the field in early autumn 1958, one female lived 33 days; and at 50–60°F. in the laboratory, one female lived 40 days. In the laboratory, the female parasites fed upon honey, and high mortality resulted within one or two days if this food was not available.

TABLE I.

Expansion of the egg of *P. falcatus* with time after laying, at 74°F. (Measurements in microns.)

No. eggs	Age in hours	Average length less pedicel	Average width	Average increase in size (¹)
10	Ovarian eggs	210.0	77.0	1.0
4	15–24	218.0	86.5	1.3 X
4	30–48	291.8	110.0	2.8 X
3	63–72	505.7	188.0	14.4 X
4	88–96	744.5	299.0	53.5 X
8	98–120	1142.6	430.1	169.8 X
3	132–141	1147.7	464.3	198.7 X

¹ X = times mean ovarian size; volume calculated from $V = \frac{\pi B^2 A}{6}$ where B is the diameter and A the length of the egg.

Development of the immature instars.

The egg is moved by the haemolymph of the weevil and finally comes to rest in the central part of the abdomen. Its growth at 74°F. is rapid. The expansion of the egg at 74°F. is illustrated in fig. 1 and Table I.

The host weevils were exposed to the parasites for about four hours, and this period is included in the age of the eggs. The maximum size observed was 1411 μ long and 548 μ wide; this egg was 121 hours and 50 minutes old, including a two-hour period of exposure of the host to the parasite. Its volume (less pedicel) compared with that of the average ovarian egg (less pedicel) showed a 340-fold increase. This is considerably less than that reported for other Braconid endoparasites: Jackson (1928) reported an increase of 1,200 times for the egg of *Perilitus rutilus*, Smith (1952) an increase of 1,190 times for *Microctonus vittatae* Mues., and Ogloblin' (1913) an increase of 1,000 times for *P. coccinellae* (Schr.).

The first-instar larva is found in the haemocoel four to six days after deposition of the egg; the majority of larvae remain within the trophamnion until the fifth day. The mature embryo is active, and the head, abdomen and tail are flexed with sustained, vigorous writhing movements that push the sac-like trophamnion one way, then another. The discrete cells of the trophamnion are mature when the embryo is mature. They may dissociate at this stage without larval activity, or eclosion might result from a break in the trophamnion.

Unless diapause intervenes, the development of the first to fifth larval instars is completed in 11 to 12 days at 74°F. Development from deposition of the egg to emergence of the final-instar larva is completed in 15 to 16 days. In 1959, in field cages, development was completed in 21 to 28 days in late May and June and in 14 to 20 days in July.

At 74°F., the maximum time of development of the non-diapause first-instar larva was one to two days. The first larval instar is a critical stage in the development of the parasite. At this stage, development may proceed without interruption or it may be arrested by diapause. As only one larva develops to maturity, it is in this stage that supernumerary parasite larvae die. The time required for the development of the second-instar larva at 74°F. was estimated at two to three days; for the third instar, three days; and for the fourth instar, four days. The majority of fifth-instar larvae left the host on the 16th day after deposition of the egg.

The beginning of the final larval instar, the fifth, is marked by emergence of the larva from the weevil. The larva moves its body back and forth in spinning motions before emergence is completed. Contact with a surface is made first by the labial snout and then by the terminal abdominal segment which grips the surface during the spinning of the cocoon. The larva does not leave the point of emergence, and the host weevil may be caught and held by strands of silk. The formation of the cocoon is begun immediately and continues without rest for 16 to 21 hours at 74°F. This period of development of the fifth-instar larva from emergence to the prepupal instar varied from 21 to 24 hours and is in addition to the period in the host when the larva is enclosed by the cuticle of the penultimate larval instar. Soon after the cocoon is completed, the larva assumes the form and quiescent habit of the prepupa. At 74°F., the prepupa developed in about 24 hours. The end of the developmental period is marked by the appearance of the cast larval skin on the inner surface of the cocoon. The cast skin is adjacent to the waste material of larval metabolism which was voided by the prepupa.

The adult emerged from the cocoon in 7-8 days at 74°F. in the laboratory, in field cages in early June 1959 in 10-11 days, and in late July and early August in 8-11 days. The circumference of the anterior end of the cocoon is neatly cut, leaving a loosely-attached cap.

The effects of superparasitism and multiparasitism.

Supernumerary larvae are affected in the first instar, soon after eclosion. The first larva to hatch is not necessarily the survivor, as older first-instar larvae were sometimes found to be affected by younger larvae or the egg. There was no evidence to suggest that supernumeraries are eliminated by combat. Dead larvae are shrunken and transparent in appearance or partially melanised. Other supernumerary first-instar larvae appeared to be paralysed but judged from the yellow colour of the mid-gut had ingested food. In a few larvae the cuticle was lightly spotted with melanin and the abdomen was abnormally twisted. Larvae may survive in this condition with normal advanced-instar larvae and were found in dying weevils from which final-instar larvae had emerged.

The interaction between first-instar larvae of *Pygostolus falcatus* and *Perilitus rutilus* in *S. cylindricollis* was observed in 29 multiparasitised weevils. The survivor in 93.1 per cent. (27) of the weevils was *P. rutilus*. The larvae of *Pygostolus falcatus* were affected in the same way as supernumerary larvae, and the cause of their deaths may have been the same. There was no evidence of combat between the species.

Effects of parasitism.

The first, second and third larval instars are primarily haemophagous and have no effect on the fat-body of the weevil. Possibly protein substances are absorbed through the cuticle, as reported by Schneider (1950) for the endoparasitic Ichneumonid, *Diplazon fissorius* (Grav.). The fourth-instar larva consumes the fat-body and the remainder of the haemolymph. It completely occupies the haemocoel of the living weevil, and the reproductive and digestive systems are flattened beneath it or crowded to one side. The larva is capable of vigorous movement and may change position and direction in the haemocoel. At maturity, the fourth-instar larva presses against the dorsum of the posterior segments. The pressure and the changes in position of the larva are revealed by distention and movement of the head and prothorax of the weevil. The larva penetrates the cuticle of the weevil through the intersegmental membrane between the fifth and sixth tergites. The cuticle of the fourth-instar larva is cast as the final instar emerges from the weevil. The exit of the larva is unhurried and is not hampered by the elytra of the weevil, which are relaxed.

The testes of the male weevil were not noticeably affected by parasitism. Parasitised males copulated and probably impregnated the females as their seminal vesicles contained active spermatozoa. On the other hand, the female reproductive system was eliminated by the effects of parasitism. The terminal chamber of each ovariole was distended and pale yellow in colour at its apex and was reduced in length. Eggs in the oviduct were also pale yellow and shrunken, with the yolk mass separated from the chorion. Cessation of oviposition and degeneration of the ovaries and eggs within the oviduct are brought about by the egg stage of the parasite. The oviposition of field-collected females of *S. cylindricollis* stopped within one or two days after deposition of the parasite egg. Such weevils laid few or no eggs (average 1.8), whereas non-parasitised weevils of the same group oviposited continually and laid an average of 414 eggs in 23 days. Oviposition of weevils of the summer generation of *S. leprieuri* was affected identically. The onset of oviposition in summer generations of *S. leprieuri*, *S. hispidulus* and *S. scissifrons* was prevented by parasitism by *P. falcatus*.

The weevil host is active up to and during emergence of the parasite larva. However, after the emergence the movements of the weevil are lethargic, one or more legs may be paralysed, and it does not feed. Death occurs from three to 24 hours later. Referring to the parasitism of *Barynotus moerens* (F.) by *P. sticticus*, Allen (1948) stated "Neither before nor after the larvae had made their exit did the beetles exhibit the slightest sign of injury or discomfort."

flavescens
flavescens

Diapause.

The development of many first-instar larvae was arrested in the host species of *Sitona*. This diapause occurred in the larvae at 74°F. and in the field both in weevils that had overwintered and in those of the succeeding generation which emerged in the summer.

The larvae in diapause in overwintered weevils were always eliminated by natural mortality of the host. At 74°F. in July 1956, 82.8 per cent. (58 individuals) of the first-instar larvae failed to develop in *S. cylindricollis* and in July 1957, 65.6 per cent. (118). Many larvae in *S. scissifrons* and *S. hispidulus*, in the European species *S. lineatus* and *S. humeralis*, and in the Japanese species *S. japonicus* also failed to develop.

Weevils that emerge as adults in summer hibernate during the winter and, within them, the parasite larvae in diapause. A greater proportion of parasite larvae in diapause were found in weevils of the current summer generation than in overwintered adults from the previous year. In 1958, 100 per cent. of the parasite larvae in weevils which had developed during the summer remained in diapause at 74°F. and, in 1959, 98 per cent.

The incidence of diapause of parasite larvae in field cages from June to October 1959, in *S. cylindricollis*, *S. hispidulus*, *S. scissifrons* and *S. flavescens* was comparable to that in the laboratory at 74°F. In one instance only was none of the progeny of a female retarded by diapause. The female had emerged on 27th May 1959, in the field, from an overwintered adult of *S. cylindricollis*. It was placed with a number of adults of *S. scissifrons* and 22 parasite larvae emerged from these. Subsequent dissections revealed no larvae in diapause in the surviving weevils. The adult parasites that resulted from the 22 larvae emerged in early July and parasitised a large number of overwintered adults of *S. scissifrons* and *S. cylindricollis*, but all progeny from these females remained in diapause in their first larval stage.

Diapause was not broken by periods of exposure of one to seven months at 30–40°F., as first-instar larvae failed to develop after such treatment when removed to a temperature of 74°F. However, the diapause of many larvae was broken in weevils overwintered in the field from August 1958 to February 1959. Of 45 overwintering parasitised weevils brought indoors in February and placed at a temperature of 74°F., 62.8 per cent. yielded parasite larvae. The percentage development in a similar lot of weevils held under variable greenhouse conditions was 55.3.

Trophamnion and teratocytes.

The function and biology of the trophamnion and teratocytes were discussed by Hinton (1954) with reference to the work of Jackson (1928, 1935) on *Perilitus rutilus*. The trophamnion is a single layer of cells immediately beneath the chorion that envelops the embryo. When the larva hatches, the cells dissociate into the haemolymph of the host and are then termed teratocytes (Hollande, 1920). The trophamnion of *Pygostolus falcatus* is differentiated early in the development of the egg. The cells increase in size with age: at 15 hours after deposition of the egg they are 5 μ in diameter; at 46 hours, 11 μ ; at 63 hours, 18 μ ; at 88 hours, 23 μ ; and at 120 hours, 32 μ . They have large, granular nuclei and are somewhat hexagonal in shape. Before dissociation, the mature cells become spherical and when the larva hatches they separate from one another and come to rest without order among the internal organs and on the floor of the haemocoel of the host. The teratocytes of the trophamnion of *P. falcatus* do not float in the haemolymph, as reported by Smith (1952) for *Microctonus vittatae*. At the time of dissociation, the cells measure 40–45 μ in diameter. They quickly lose their transparent, granular appearance and become uniformly white and opaque.

The teratocytes are distinct and positive indicators of parasitism. They are concentrated along the length of the abdomen of the weevil, but a few of them may be carried into the prothorax by the haemolymph.

The teratocytes of most of the first-instar larvae of *P. falcatus*, 6 to 15 days of age, did not exceed 90μ in diameter and this maximum size was reached within one or two days after eclosion. Cells of this size were found in weevils overwintered in the field. On the other hand, their maximum size associated with diapause larvae in current-season adults of *S. cylindricollis* maintained at 74°F . for 177 days was 203μ . Teratocytes as large as the latter were the exception, as with them were numerous cells 70 to 90μ in diameter. The number of teratocytes associated with first-instar larvae varied greatly. The maximum number in a host containing one six-day-old larva only, was 747; the minimum number was zero, an instance where the trophamnion had failed to dissociate. Other cell counts with the age of the larva in days in parentheses are: 669 (7); 399 (6); 433 (6); 307 (6); 525 (6); 390 (15); 16 (14); 10 (19); 450 (177); 135 (177); 210 (177) and 480 (193).

The teratocytes invariably increased in size in association with advanced-instar larvae. There was, however, considerable range in size between the cells of any one larva, and some showed no expansion whatever. The maximum size of cells of second-instar larvae 6 days old was 128μ ; third-instar larvae 9 and 10 days old, 240μ ; third- and fourth-instar larvae 11 days old, 340μ . These large cells are eventually crushed by the larva which at maturity occupies all of the haemocoel. Thus the teratocytes of some fourth-instar larvae 12 to 14 days old were smaller, 240μ maximum, than those associated with larvae 11 days old; and of 15-day-old larvae the maximum was 170μ . The number of teratocytes associated with advanced-instar larvae accordingly decreased as the larva matured. Larvae 14 and 15 days old were found with 8 to 70 cells; however, 265 and 280 teratocytes were counted in two dissections of similar age. The weevil from which the fifth-instar larva emerged usually contained a small number of cells in the range 51 to 90μ .

Field history.

The seasonal history of *P. falcatus* was worked out at Belleville from the data of intermittent collections of *Sitona* spp. in Sweden in 1956 and 1957 and from regular collections in Sweden in 1958.

The host weevils form a complex of species of *Sitona* associated with various species of economic legume plants. It is probable that any species of *Sitona* may be parasitised by *P. falcatus* in the field as an oviposition preference was not evident in the laboratory. The primary host species in the mass collections from the Kattarp area of Sweden was *S. lineatus*. *S. humeralis* predominated in one collection only, obtained in early August 1957. The weevils were swept in 1957 from vetch, *Vicia sativa*; in 1958 from alfalfa, *Medicago sativa*, intermixed with a slight proportion of red clover, *Trifolium pratense*, and from a field of peas, *Pisum arvense* and *V. sativa* (private communication, S. Johansson).

Wagn (1954) studied the seasonal abundance and distribution of *Sitona* species at Virumgaard, Lyngby, Denmark. *S. decipiens* Lindberg was the dominant species in his collections on alfalfa in spring and early summer; in decreasing order of abundance were *S. humeralis*, *S. lineatus*, *S. hispidulus*, *S. puncticollis* Steph. and *S. sulcifrons* (Thnb.). On red clover and white clover, *T. repens*, *S. hispidulus* was most abundant, and on alsike clover, *T. hybridum*, *S. flavesceus*.

Pygostolus falcatus overwinters as first-instar larvae in weevils which became adult in the previous summer. The larvae develop in the spring and probably emerge in late April and May. Adults that develop from the overwintered larvae are active in June and parasitise others of the weevils that have overwintered. Development is continuous and rapid (see Table II) and the succeeding parasite generation emerges in late June and early July. Weevils of various *Sitona* species

also reach the adult stage in July, or later, and are then attacked by adults of this mid-summer generation of parasites. The development of the majority of the resulting first-instar larvae is arrested, and the larvae remain in diapause for the remainder of the field season, and through the following winter.

TABLE II.

The incidence and succession of the immature instars of *P. falcatus* in 1958 in *S. lineatus* in the field.

Date of collection	No. weevils		Percentage parasitism		
	Dissected	Parasitised	Egg	First instar	Advanced larvae
1958					
27-29 May ..	101	0	0	0	0
6-7 June ..	105	2	0	1.9	0
12-14 June ..	197	1	0	0.5	0
21-24 June ..	100	50	4.0	42.0	4.0
27-28 June ..	90	66	3.3	61.1	8.9
2-4 July ..	100	50	0	6.0	44.0

First-instar larvae were scarce in or absent from collections made in late May and early June. The few recovered in dissections may have been in diapause from the previous year. The parasitism of 21st June and 27th June was marked by a sharp increase in numbers of first-instar larvae, the result of emergence and oviposition activity of the overwintered parasite generation. The sudden decrease in first-instar larvae in early July is an expression of adult parasite mortality and of development of the advanced-instar larvae. No data were obtained after 5th July 1958, as the weevils were too scarce to collect (private communication, S. Johansson). This decrease of the overwintered generation of the weevil could coincide with emergence of the parasite larvae.

The mid-summer parasite generation emerges after the population of overwintered weevils has been depleted by parasitism and natural mortality. Percentage parasitism of the current generation of the weevil, that had developed during the summer, was consistently low in the autumn collections of weevils. In late September 1956, six per cent. of *S. lineatus* and *S. hispidulus* were parasitised; in August 1957, less than one per cent. of *S. humeralis* were parasitised; and in late September 1958, 16 per cent. of *S. lineatus* were parasitised. This low rate of parasitism may be a result of poor synchronisation between the parasite and host or of decimation of the mid-summer parasite generation by natural enemies and weather. Poor synchronisation may be the main factor: Wagn (1954) found considerable variation in the periods of peak abundance of adults of the current-summer generation of weevil species on alfalfa.

Importance as a control agent.

P. falcatus has three important attributes as a control agent of *Sitona* populations: its parthenogenetic, thelytokous reproduction; its non-specificity of hosts within the genus *Sitona*; and a tolerance of extremes of low temperature. In addition, the parasite species need not be abundant to achieve a high incidence of parasitism. A 73.3 per cent. parasitism at Kattarp in 1958 developed from an overwintering larval population in less than one per cent. of the weevils. The almost immediate cessation of oviposition by the parasitised female weevil may in some instances be an important factor in the reduction of the host population.

Detracting from the success of *P. falcatus* as a control agent in Sweden is the low rate of parasitism of the overwintered weevils in the spring and early summer when they oviposit and are most injurious to legume crops. This may result from poor synchronisation between the emergence of adults of the mid-summer parasite generation and the new generation of weevils and it is maintained by diapause of the first-instar larva that overwinters. The overwintered weevils are therefore not reduced by parasitism until they are near the end of their normal life-span.

Summary.

Pygostolus falcatus (Nees), an endoparasitic Braconid of the subfamily BLACINAE, was reared from *Sitona lineatus* (L.), *S. hispidulus* (F.) and *S. humeralis* Steph. of Swedish origin. The life-history and immature stages of *P. falcatus* were studied in the laboratory at 74°F. and in field cages preparatory to its liberation in Canada as a control agent of the sweetclover weevil, *S. cylindricollis* Fhs.

The immature stages develop in the haemocoel of the weevil. After deposition, the egg increases in volume to a maximum of 335 times. At eclosion, the cells of the trophamnion dissociate and increase in volume in relation to the growth of the larva. Supernumerary larvae are eliminated soon after eclosion, and one larva only develops in a host weevil. At 74°F., the final-instar larva emerges from the host weevil 15–16 days after deposition of the egg. Development of many first-instar larvae in a wide range of *Sitona* species was arrested by diapause. Five larval instars are described and illustrated. Oviposition is prevented in weevils in the summer of their development and eliminated in overwintered weevils by the parasite egg and first-instar larva. The weevil dies within hours of emergence of the parasite larva.

Reproduction is parthenogenetic and thelytokous. The maximum number of eggs laid by a female was 46; the number of eggs laid per day per female varied from 0 to 11. Oviposition takes place both in light and in complete darkness and a preference among species of *Sitona* was not evident. The maximum number of weevils parasitised by a female in a field cage was 39; the number parasitised per day varied from 0 to 8.

The species overwinters as a first-instar larva in weevils which became adult in the previous summer. There were two discrete parasite generations in 1958 in Swedish weevils, both of which developed in early and mid-summer within overwintered weevils. The maximum rate of parasitism of *S. lineatus* by larvae of the mid-summer parasite generation in 1958 was 73.3 per cent.

The value of *P. falcatus* as a control agent of *Sitona* populations is limited by low parasitism of the new weevil generation of the current year and diapause of the first-instar larva.

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THE CONTROL OF THE COCONUT PEST *MELITTOMMA INSULARE* (COLEOPTERA, LYMEXYLIDAE) IN SEYCHELLES.

By I. W. B. NYE

Commonwealth Institute of Entomology.

The larva of the Lymexylid beetle, *Melittomma insulare* Fairm., has been recognised as a pest of coconut palms in Seychelles since the beginning of this century. Frequent references to it, and suggested control measures to combat it, subsequently appeared in the reports of the Department of Agriculture, but the first detailed account of the biology and incidence of attack of this insect was given by Vesey-FitzGerald (1941).

During the following ten years, the damage in some islands, particularly Praslin, increased to such an extent that further technical assistance was requested and as a result Vesey-FitzGerald revisited the islands in 1952 accompanied by Dr. F. J. Simmonds and E. S. Brown. Simmonds, who was studying the possibility of biological control, stayed for a few weeks and then continued the search for beneficial insects in Mauritius, East Africa and Trinidad. He gathered together information concerning the world species of LYMEXYLIDAE and wrote that he considered several of them might warrant further investigation with a view to providing natural enemies for use against *M. insulare*, but, in general, what was known of their biology did not afford much hope that an effective parasite or predator would be found (Simmonds, 1956). He considered that the most likely possibility was *Rhizophagus dispar* (Payk.), a Nitidulid beetle which is predacious on the eggs of another Lymexylid beetle, *Hylecoetus dermestoides* (L.), occurring in Britain. Several consignments, mainly of *R. dispar* but also including *R. ferrugineus* (Payk.), *R. depressus* (F.), and *R. bipustulatus* (F.), were received and liberated in the islands of Mahé and Cerf during 1955 (Lionnet, 1959). A survey of the areas where they were released has failed to reveal their presence and it seems unlikely that any of them have become established.

Brown remained for a year in Seychelles and subsequently, in 1954, published a comprehensive account of the biology, incidence and control of *M. insulare*. In 1953, the Government of Seychelles started a pilot scheme for the maximum control of the pest in the island of Praslin. This, the second largest island of the group, has an area of 15 sq. miles and lies 23 miles north-east of the main island of Mahé. All the 90,484 coconut palms in Praslin and the 5,665 in the small adjacent island of Curieuse had been examined and, if necessary, treated by the beginning of 1958 and it was then considered advisable for an entomologist to examine the results of the pilot scheme before the treatment of Mahé and its offshore islands was commenced.

The present paper is based on two departmental reports which have been issued on the control of *M. insulare* in Seychelles (Nye, 1959, 1961).

Biology.

The slender, dark-brown adults of *M. insulare* vary in length from 8 mm. to 18 mm. They are poor fliers and have an extremely short adult life, rarely longer than a week in laboratory conditions. The females lay their eggs on coconut and other palms in cracks of the trunk, usually at its base. When the

larvae hatch out they bore into the bole, and travel inwards and usually upwards into the softer central tissues for at least a year. These larvae daily back down their tunnels, pushing the accumulated chewed fibrous residues and frass out of the entrances by means of a sclerotised circular tailpiece with a serrated edge. At the same time the serrated tailpiece is used to enlarge the diameter of the passage after each of the frequent larval moults.

In advance of and surrounding each of the tunnels is a zone of wood infected by bacteria, and other micro-organisms associated with the damaged tissue. These diseased zones gradually spread and coalesce, particularly in the softer, moister central part of the trunk where there are relatively fewer vascular bundles. Although the mechanical damage to a palm resulting from the tunnels alone is very slight, the diseased zones affect a much greater volume of tissue. This never recovers and gradually decays until eventually an empty cavity is left in the base of the trunk.

As palms age, their trunks become harder. When older palms are attacked, the central cavity formed gradually becomes larger and the peripheral ring of hard sound wood at ground-level is slowly weakened by further larval infestations and the consequent spread of rot, until the palm eventually falls. This may not occur for upwards of 20 years. In some cases, decline may be retarded indefinitely, as when, for example, free circulation of air in the hollow dries out the tissues and so retards or halts the spread of rot and at the same time discourages new basal infestations. In the case of younger palms of up to 25 years old the boles are soft and sappy. When these are attacked the rot spreads rapidly and extensively. The young palms then fall after about five years, before reaching the hollow stage.

Once a palm is attacked, it remains infested with larvae for the rest of its life, until it falls. Palms in which an infestation has died out are extremely rare. However hollow a palm may be, the infestation continues, albeit progressively more slowly, higher and higher up the centre. Once an attacked palm reaches the hollow stage, new roots are often put out from the trunk above ground-level. Additional adventitious roots are formed higher up the trunk as the infestation progresses, frequently up to about two feet above the level of the surrounding soil. These root masses form ideal oviposition sites, giving rise to more basal attacks which mechanically weaken the palms.

In any plantation, even one over 40 years old, there are some palms which are completely free of any infestation by *M. insulare*, some which are newly and therefore superficially infested, some deeply infested, some hollow and some already fallen. Palms at all stages of attack can be adjacent to one another and entirely uninfested palms are invariably to be found intermixed with, and frequently only 25 ft. or less from, highly infested, hollow, or fallen palms. In the case of older plantations, many of the infested palms must have continuously harboured successive generations of up to about 250 larvae over the past 20 years. This emphasises that the rate of spread from palm to palm is slow and that there must be considerable mechanical resistance by older palms to attack. The fact that superficial infestations of recent origin can always be found in some of the old palms shows, however, that their resistance is not absolute.

Adult beetles emerging from a palm seem to remain close to it, possibly being attracted by the characteristic odour of the diseased wood. It seems probable that the larvae within a trunk are the descendants of those which caused the original infestation. When the host palm falls, the trunk starts to dry out, ceases to produce the characteristic odour and apparently no longer provides suitable oviposition sites. The adults which still continue to emerge for about three months then have to disperse to adjacent living palms. There is a tendency for a greater proportion of superficial infestations to occur in palms adjacent to one which has recently fallen.

The 1953-1958 PDCB treatment of Praslin.

The method of control used during the pilot scheme in Praslin was based on that suggested by Brown (1954, pp. 47-53) who experimented with paradichlorobenzene (PDCB), a white crystalline solid which slowly gives off a vapour toxic to larvae of *M. insulare*. The infested area of the trunk must be gouged away to cut across all the larval tunnels so enabling the vapour to come into contact with the larvae.

For superficial infestations, the surface wood was shaved off and about 250 g. of paradichlorobenzene were placed in the cavity so formed. Coconut husks were then packed tightly around the hollow to a level above the uppermost tunnel opening and soil to a depth of a foot was compacted over the husks so as to form a fumigation chamber. In order to protect this, a retaining wall of coconut husks usually had to be built around the palm and filled with earth. For deep infestations penetrating over six in. into the trunk the procedure was the same except that all dark-brown rotted wood was removed and up to twice as much insecticide was used.

Palms which were supported by so little sound wood that they could not be treated, or which fell during or soon after PDCB treatment, were cut into three-ft. lengths and stacked. The bole was split up so that it would dry out more rapidly and so prevent further development of the larvae of *M. insulare* within it. After about a year, the stacked logs had dried out sufficiently and they were then split and burnt in order to prevent them from becoming breeding sites for rhinoceros beetles.

Owing to the large amount of labour involved, particularly in walling up around fumigated palms, and disposing of fallen palms, the costs were high and the total financial outlay of £12,150 when divided between the 96,149 coconut palms examined and treated in Praslin and Curieuse worked out at 2s. 6d. per palm; or when divided into the 77,011 palms in Praslin and 5,488 in Curieuse still standing a week after PDCB treatment, 3s. per palm.

The records obtained during the 1953-1958 PDCB treatment of Praslin are summarised in the upper part of Table I. At the time of the treatment, out of 90,484 palms, only 23 per cent. were found to be uninfested, 25 per cent. had superficial infestations and the remaining 52 per cent. were deeply infested and hollow palms of which 14 per cent. were felled as beyond treating or fell within a week of treatment. The number of nuts over three inches in diameter borne on each palm was counted and averaged 23 per uninfested palm and 31 for both superficially and deeply infested palms. The lower average yield for uninfested palms is due to there being a greater proportion of immature trees, not in full bearing, within this group. The degree of infestation does not affect the yield. The losses caused by this pest are therefore mainly due to the palms falling during or before early maturity, thus occasioning continual replanting of seedlings, which do not come into bearing for a further seven years.

Methods of control.

An examination of the Praslin palms in April 1959 showed that the 1953-1958 PDCB treatment had been only partially effective. Many palms, even though earthed up, had fallen since treatment and the loss was continuing. On opening the fumigation chambers it was found that the inside of the palm was usually wet, and rot had continued both in the basal portion of the bole and up the centre of the trunk, even though in a few cases there was no longer any infestation by larvae of *M. insulare*. These observations confirmed the findings of Brown (1954, p. 15) that once established in the trunk of a palm, the micro-organisms causing the breakdown of palm parenchyma continued to spread even in the absence of larvae of *M. insulare*.

Later in the same paper (pp. 45-60) Brown has reviewed the historical methods of control and described experimental trials using paradichlorobenzene. On a field scale this latter method of treatment has since been shown to be unsatisfactory for the following reasons:— (i) The closed fumigation chambers continue to act as a focus for the spread of further rot in the trunk; (ii) In the majority of fumigated palms an incomplete kill was obtained and the progeny of

TABLE I.

Summaries of the records obtained during the 1953-1958 PDCB treatment and the 1959-1960 tar retreatment of Frasin.

	Number of palms	Percentage of palms	Number of nuts	Average number of nuts per palm
1953-1958 PDCB treatment				
Uninfested palms	18336	23	414000	23
Superficially infested	23317	25	718000	31
Deeply infested	35358	38	1087000	31
Palms falling during or within a week of PDCB treatment	13473	14	—	—
Total	90484	—	2219000	29
1959-1960 tar retreatment older palms				
Uninfested palms	27616	31.0	—	—
Superficially infested	6708	7.5	—	—
Deeply infested	29195	32.0	—	—
Palms falling during or within a week of tar retreatment ..	455	0.5	—	—
Palms already fallen as a result of PDCB treatment ..	26510	29.0	—	—
Total	90484	—	1858000	29
1959-1960 tar retreatment younger palms				
Uninfested palms	7648	66.0	—	—
Superficially infested	2748	23.5	—	—
Deeply infested	1176	10.0	—	—
Palms falling during or within a week of tar retreatment ..	55	0.5	—	—
Total	11627	—	87000	8

the survivors continued the infestation; (iii) It was not possible to diagnose these residual infestations without opening all the chambers; (iv) In order to protect the fumigation chambers of deep infestations it was essential to build a retaining wall one to two feet high using up to 250 husks, and to fill the enclosure with soil; and (v) Although some beneficial effect on the palm resulted from the fresh soil around its base encouraging the formation of new roots, earthing up was useless if the wall was not maintained and, under the prevailing conditions of heavy rainfall, the soil was eroded and the newly formed roots left exposed.

Accordingly, all control methods were considered with the object of eliminating these disadvantages and at the same time attaining the following main objectives:— (i) The highest kill of larvae of *M. insulare*; (ii) the prevention of spread of rot in the trunks after elimination of larvae of *M. insulare*; (iii) the least

mechanical and physiological damage to the palm; (iv) the certainty and ease of locating and killing larvae which survive a first treatment; (v) the reliability, simplicity and low cost of treatment.

External application of fire, tar, lime, ashes, common salt and insecticides, without gouging out diseased tissues, have been tried in the past and were at their best only partially effective. The larvae live right inside the trunk and back down to the entrance of their tunnels pushing out frass and detritus by means of their tailpieces. Once a hollow is formed by the rotting of the bole, many of the entrances to tunnels are on the inside and never appear on the external surface of the trunk. It is therefore difficult to envisage any improvement in technique which will make any such external application more effective as a treatment.

Fumigants requiring an internal fumigation chamber and walling up are open to the same objections as paradichlorobenzene unless one can be obtained which will give a complete kill of larvae together with strong bactericidal and fungicidal action. Quite apart from any other considerations, any method involving the use of large quantities of husks is expensive in labour and materials. Owing to their use in cinnamon-leaf distilleries, copra driers and homesteads as fuel and their greatly increased use during the past few years as a mulch in valuable vanilla plantations, husks in extensive areas are in very short supply and have a market value of about a shilling a hundred.

Another possible method of control is by external fumigation by means of a gas such as a methyl bromide introduced into a small polythene tent secured round the base of the palm. Dr. A. B. P. Page and Dr. O. F. Lubatti very kindly carried out tests at Imperial College Field Station, Berkshire, England and sent all the necessary equipment to Seychelles. Trials showed that a 20-ml. ampoule of methyl bromide (the smallest size commercially available) produced a complete kill of larvae of *M. insulare* in heavily infested palms fumigated for three hours. There were no phytotoxic effects. Although external fumigation causes the least possible damage to the palms, there still remains the problem of the spread of rot in the trunks and, when used on a field scale, the uncertainty of being able to locate any surviving larvae.

Systemic insecticides were tried by Brown and were found to be unsatisfactory. This is almost certainly due to the larvae being always surrounded by necrotic tissues in which translocation is probably impeded. Because of this it is unlikely that any modern systemic insecticide would be any more successful than those already tried.

Gouging out rotted tissue and the internal application of an insecticidal wood preservative appeared to be the most promising alternative to fumigation. This avoided the need for a closed chamber, and mere exposure of the diseased wood to the air assisted in drying it out, greatly retarded the further spread of rot within the trunks and enabled the tunnels still containing living larvae to be seen easily.

Experiments were carried out using a medium-viscosity British refined coal tar and a low-viscosity Stockholm pine tar, and it was found that the degree of control achieved depended on the type of tar used.

The coal tar when applied to a freshly gouged palm trunk went on like thick paint. There was very little penetration, and the tar tended to form a seal over the area, including any necrotic tissue which had not been excised. There was thus very little preservative action and the diseased tissues were prevented from drying. In addition, the thick tar formed a small plug at the entrance to each larval tunnel, and although many of the larvae became stuck in the tar when they backed down to clear the obstruction, many others, protected by a buffer of frass, pushed out the plug of tar and remained unaffected.

When the pine tar was painted on after gouging there was rapid and almost complete penetration and although this tar crept up the tunnels it was rapidly absorbed and very little deposit was left on their walls. The larvae, on backing down, did tend to get some tar on their abdominal segments and many were killed. A second tarring applied a week after the first to 132 infested palms resulted in a further kill of larvae, but 26 per cent. of the palms still contained an average of ten live larvae.

Various mixtures were tried, and a combination of 75 per cent. Stockholm pine tar and 25 per cent. refined coal tar was found to give the most satisfactory results. There was good penetration of the tissues by the pine tar, which carried with it some of the coal tar and also left a thin surface layer which remained tacky for several days. This mixture also crept up the tunnels and the deposit that was left on their walls became transferred to the body segments of the larvae when they backed down to the entrances. Within half an hour the larvae could be seen hanging out of their tunnels, dead.

A large-scale trial was then started at Montagne Posée in Mahé. Out of 1,000 palms, of mixed age, 825 were infested. These were gouged and tarred the following day with the pine-tar/coal-tar mixture and were then retarred a week later. On re-examination, 15 per cent. of the tarred palms still contained living larvae but these only averaged three per palm. Brown (1954, p. 39) records counts of up to 255 larvae in a palm, with an average of 129 larvae per infested palm aged from 7 to 25 years and 45 larvae per infested palm aged from 25 to 70 years. The larvae develop for over a year within the palms, and, on emergence, the adults live for only about one week. They are not strong fliers and during this period they must find a mate. As already stated it seems that adults tend to remain attracted by the palm which acted as a host to them as larvae. When the number of larvae living in a palm is reduced to single figures there is little chance of a male and a female emerging from the same host palm within the same week, and many of these residual infestations will probably die out in the absence of further oviposition.

Palms should be tarred the day after gouging. During the interval the wet, freshly gouged, surface dries slightly and the larvae have time to clear their tunnels of any debris which may have been pushed into them during gouging, and thus likely to prevent the entry of the tar. After painting excised surfaces with this intensely black mixture it is easy to detect any tunnels which still contain live larvae by the light-coloured dust and particles ejected from them within 24 hours. A second application of tar mixture should be applied a week after the first, as by then any residual infestation becomes obvious.

In order to reduce the cost of treatment on a large scale it was necessary to find an alternative to Stockholm pine tar. Experiments were carried out in collaboration with the Chemical Products Department of the South Eastern Gas Board, England, who recommended a mixture consisting of high-specific-gravity coal-tar creosote blended with a refined coal tar of medium viscosity. The mobile nature and phenolic content of the mixture ensured absorption and penetration on a moist surface and the tar was such that the residue remaining on the surface after drying was soft and did not flake off.

The mixture conformed to the following specification when tested by the methods recommended by the American Wood Preservers' Association:—

Coal tar creosote	per cent. by volume	60 min.
Water	per cent. by volume	3 max.
Matter insoluble in benzene	per cent. by weight	3.5 max.
Coke residue	per cent. by weight	9.0 max.
Specific gravity at 38°C. compared with water at 15.5°C.		{ 1.13 max. 1.09 min.

Distillation:

per cent. by weight distilling up to 210°C.	5 max.
" " " " " " " 235°C.	{ 25 max.
" " " " " " " 315°C.	{ 5 min.
" " " " " " " 355°C.	32 min.
" " " " " " " 355°C.	52 min.

One gallon of the mixture (costing two shillings per gallon including container, 'free on board' at London) is sufficient to treat, with two applications, approximately five deep or 15 superficial infestations.

The weakest part of an infested palm is at ground-level, and great care must be taken to remove the minimum of sound wood from that region. There is usually at least one rotted sector which may be cut through to gain access to the centre, without causing any loss in strength, and from there the cavity must be extended upwards and inwards as shown in fig. 1, excising all black and dark-

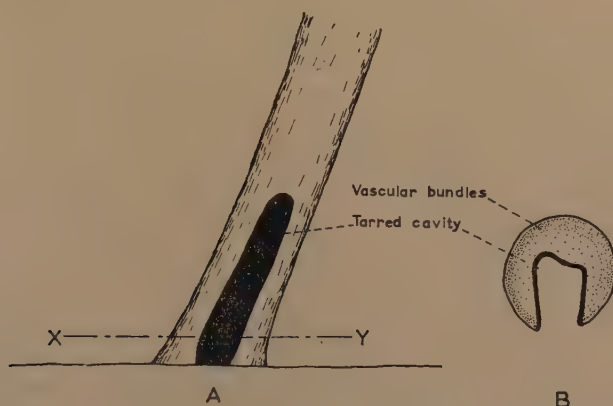


Fig. 1.—A, diagram of a palm trunk showing the tarred cavity after excision of necrotic wood; B, cross-section of the trunk at XY, showing how the excision has been made to ensure that the minimum of peripheral hard wood, with concentrated vascular bundles, is removed.

brown necrotic wood. Some of the paler-brown diseased wood should be removed, but there is no need to get rid of it entirely. If all such wood is removed then not only will all the larvae have been cut out but also, owing to the irregularity of the advance of the rot, much healthy tissue will have been unnecessarily destroyed. If insufficient necrotic wood is removed then not all the tunnel entrances will have been exposed and the tar mixture will then have no effect on those larvae. A balance must be achieved, based on practical experience, between obtaining a complete kill and possibly causing unnecessary damage to the palm, and leaving some residual infestation to be eliminated by subsequent spot treatment.

Hand-axes are used for the initial openings in the palms. For hollowing out, the most useful tool is a bar gouge, a metre-length of bar-steel flattened at one end to form a concave cutting edge about six centimetres across. For finishing and smoothing inside the cavity it is best to use a short-handled adze with an eight-centimetre, slightly concave, cutting edge.

When a palm has been attacked at more than one site at the base of the

trunk it is possible either to make two openings or, using only the one opening, to gouge through some healthy wood at the centre in order to gain access to the infested zone on the other side. The general guiding factor should then be that it is better to sacrifice some of the soft central wood which gives little mechanical support and contains few vascular bundles rather than destroy at ground-level any of the peripheral layer of healthy hard wood on which the palm depends both for mechanical support and conduction of sap.

By careful gouging of the trunk by the above method, so as to cause the minimum damage to the tough outer layer of concentrated vascular bundles, it was possible to carry out the tar treatment with an over-all loss of less than one per cent. of the total of palms. These were either too rotted at the base to withstand treatment or fell within a week of it.

The 1959-1960 tar retreatment of Praslin.

The island of Praslin was retreated during the period November 1959 to April 1960 using the tar method. The records obtained have been summarised in the lower two parts of Table I. For comparative purposes it has been necessary to separate the figures relating to the older palms, which had previously been numbered and treated during 1953-1958, and the figures relating to younger palms. These latter have been defined as having at least one foot of trunk, free from fronds, showing above ground.

The records of tar retreatment of older palms (Table I) show that as a consequence of the 1953-1958 PDCB-fumigation treatment there had been an eight per cent. improvement from 23 to 31 per cent. in the number of uninfested palms. The reduction of from 25 to 7.5 per cent. and from 38 to 32 per cent. in superficially and deeply infested palms, respectively, was due, not, as at first appears, to an improvement, but to a deterioration in their condition which resulted in the superficially infested becoming deeply infested and the deeply infested falling. This is shown by the number of fallen palms, which increased from 14 to 29 per cent. It must be borne in mind that this depletion of 29 per cent. of the island's palms over a period of seven years includes an inevitable annual loss of about 1.5 per cent. due to aging and, in addition, about five per cent. of worthless hillside palms which were felled rather than treated. Even allowing for these factors, the level of losses remained too high when the PDCB method was employed.

Of the younger palms that had never been treated but were growing amongst the PDCB-fumigated older palms, 23.5 per cent. were found in 1959-1960 to be superficially and 10.5 per cent. deeply infested. These figures confirm that *M. insulare* had not been brought under sufficient control by the 1953-1958 campaign.

There was a total of 75,601 palms standing at the time of the Praslin tar retreatment and, of these, 40,337, or 53 per cent., were still infested. The over-all cost of examination and retreatment where necessary worked out at an average of eightpence a palm or about 1s. 3d. per infested palm. A labourer's wage was 90 shillings per month and the landed cost of the tar mixture about 3s 6d. per gallon.

At the conclusion of the 1959-1960 tar retreatment, 38 samples, each of 25 palms treated from one to three months previously, were carefully examined and the number of palms still containing living larvae was recorded. These results are summarised in Table II and show that there were small residual infestations in 9 per cent. of the coastal coral-sand palms, in 14 per cent. of the coastal marsh palms and in 18 per cent. of the hillside palms. Direct comparison with percentage infestation figures recorded at the time of the tar retreatment and shown in Table I is not possible since the latter are worked out from totals that include a large proportion of palms, particularly in the older group, that had already fallen, whilst

the samples in Table II were taken on the mixture of older and younger palms actually standing. Of these, an average of 53 per cent. had been infested (see above), and this figure is comparable. The higher residual infestations in the coastal marshes and hillside reflect the greater initial infestations and difficulty of treatment in these locations.

TABLE II.

Results of samples taken at the conclusion of the 1959-1960 tar retreatment of Praslin.

	Coastal coral sand	Coastal marsh	Hillside
Number of palms examined	375	275	300
Uninfested palms (%)	91	86	82
Tar-treated palms still containing live larvae (%)	9	14	18
Average number of live larvae per residually infested palm	4	8	4
Palms with new untreated infestations ..	0%	1%	0%

The tar treatment of Mahé.

Control measures against *M. insulare* using the tar method were commenced on Mahé, the largest island of the group, in April 1960. Ten teams were deployed throughout the island to examine and treat, at the rate of 200,000 per year, all of the estimated million coconut palms. Up to the end of December 1960, out of 152,000 palms examined, 80 per cent. had been found to be infested and had been treated. This infestation is greater than the two earlier estimates of 75 per cent. (Nye 1959, p. 30) and 32.58 per cent. (Brown 1954, p. 34).

To assess the results of this technique when applied on a field scale to palms to which no previous attempt at control had been made, samples, each of 20 palms, were re-examined about six months after tar treatment. Five samples of palms growing in each of the three main habitats, coastal coral sand, coastal marshes and hillsides, were recorded from five localities around Mahé, making a total of 500 palms for each of the three habitats. The percentage infestation in each of these habitats at the time of tar treatment is compared in Table III with the infestation found in the same palms about six months later. In the coastal coral-sand areas, treatment had reduced the percentage of palms infested from 74 to 13, containing an average of only 4.2 live larvae per palm. In the coastal marshes, 17 per cent. of treated palms were still infested, containing an average of 5.4 larvae, and on the hillside, where the initial infestation was 83 per cent., there were 24 per cent. of palms containing an average of five larvae each.

Of the 1,500 palms sampled, only 262 still contained living larvae which survived the treatment and, of these, 193 palms contained five larvae or less; 37 palms contained between six and ten larvae and 32 palms contained between 11 and 20 larvae. These few remaining larvae could easily be killed by blocking their tunnels with a long thorn from wild *Citrus* trees or by gouging and retarring. As already stated, when the number of larvae living in a palm is reduced to single figures there is little chance of a male and female emerging from the same host palm within the same week, and many of these residual infestations may be expected to die out.

In addition to the residual infestations in tar-treated palms, nearly two per cent. of the palms had a new untreated infestation, arising from eggs or very small larvae which were missed at the time of treatment. Such new infestations are very superficial and take little time to gouge and tar.

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These results show that a single treatment (gouging with application of tar mixture the following day, and a further application of tar mixture a week later) by trained teams, followed up by a further examination and spot treatment of residually infested palms about six months later, can reduce a very serious infestation almost to the point of eradication. Thereafter, an annual examination and

TABLE III.

Comparison of the percentage infestations at the time of tar treatment of Mahé and of the same palms about six months later.

Coastal coral sand				Six months after treatment			
At tar treatment				Uninfested palms	87%
Uninfested palms	26%	Tar-treated palms still containing live larvae			13%
Superficially infested palms			32%	Average number of live larvae per residually			
Deeply infested palms	..		42%	infested palm	4.2
				Palms with new untreated infestation	..		1%
Coastal marsh				Six months after treatment			
At tar treatment				Uninfested palms	83%
Uninfested palms	26%	Tar-treated palms still containing live larvae			17%
Superficially infested palms			36%	Average number of live larvae per residually			
Deeply infested palms	..		38%	infested palm	5.4
				Palms with new untreated infestation	..		3%
Hillside				Six months after treatment			
At tar treatment				Uninfested palms	76%
Uninfested palms	17%	Tar-treated palms still containing live larvae			24%
Superficially infested palms			29%	Average number of live larvae per residually			
Deeply infested palms	..		54%	infested palm	5.0
				Palms with new untreated infestation	..		1%

and a formulation of coal tar creosote and coal tar liberally applied. Praslin Island was retreated, using this tar method, and a plan to treat all the coconut palms in Mahé, the main island of the group, was subsequently commenced. Losses of palms during and within a week of treatment have been reduced to less than one per cent., and results are quoted which show that a single treatment can reduce a serious infestation in which 80 per cent. of palms are more or less heavily attacked to one in which only 18 per cent. of the palms are attacked and, on average, contain only five larvae. The treatment ensures that the entrances to the tunnels of these are exposed to view, and accordingly the surviving larvae can easily be killed.

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A MORPHOLOGICAL STUDY OF VARIATION IN *TYROPHAGUS*
(ACARINA), WITH PARTICULAR REFERENCE TO
POPULATIONS INFESTING CHEESE.

By PHYLLIS L. ROBERTSON

E.M.V.

*Phyllis Anderson Research Fellow, School of Public Health
and Tropical Medicine, University of Sydney.*

Mites now assigned to the genus *Tyrophagus* Oudemans, 1924, have been known as cheese pests in many countries from the time of Gervais's description of 1844 up to such present-day records as those of Hughes (1948) for the United Kingdom, Rice (1948) for Australia, Bollaerts & Breny (1951) for Belgium, and Baker (1954) for the United States. At first only one world-wide species was thought to be involved, which was identified as *longior* Gerv., 1844, and was included in the genus *Tyroglyphus* of Latreille, 1796, recently declared invalid by the International Commission on Zoological Nomenclature (Hemming, 1958). These names were used consistently until 1924, when Oudemans erected the genus *Tyrophagus* and revived Hermann's (1804, p. 85, Pl. VI, fig. 4) name *dimidiatus* for the species. Slight geographical differences were noted (Oudemans, 1906) between populations of this species described by Berlese (1884) from Italy, by Michael (1903) from the United Kingdom and by Oudemans himself from the Netherlands, New Guinea, Germany, Italy and France. In 1924, Oudemans also drew attention to habitat-related form variations within the species, and in 1929 Vitzthum described three of Oudemans's habitat "forms," including one from cheese, as varieties of *dimidiatus*. Subsequently, both the species names *longior* and *dimidiatus* were used for *Tyrophagus* infesting cheese, while still further names were introduced, such as *putrescentiae* (Schränk, 1781), *castellanii* Hirst, 1912, and *tenuiclavus* Zakhvatkin, 1941 (Womersley, 1941; Dowling & Thomas, 1942; Hughes, 1948; Bollaerts & Breny, 1951), although little attempt was made to relate one name to another. Thus, on a world-wide basis, literature offered no clear conception of the specific composition and relationships of the populations of *Tyrophagus* infesting cheese. It could only be inferred that cheese is liable to attack either by one habitat form of a single species of *Tyrophagus*, or by several geographical forms of a single species, or by a number of geographically separated species. An explanation for variation between populations might well be found in the latter suggestions, although they could not explain variation within populations.

But during investigations of the mite complexes on cheese, first in New Zealand (Robertson, 1946) and later in the United Kingdom (Robertson, 1952), note was made of the extent of variation within populations of *Tyrophagus*. There were comparatively large differences in adult size, in the relative proportions of body and legs, and in certain dorsal hair relationships. Distinct morphological types could not be distinguished with certainty, but it appeared that the over-all characteristics of a population were in some way determined by the physical conditions of storage. The present investigation was undertaken in the first place to find an explanation for this variation within populations, and to account for the way in which it was affected by environment.

A comparison of cheese populations of *Tyrophagus* from New Zealand, the United Kingdom, Australia, the Netherlands and the United States showed them to differ in some of the characters which varied within populations. The second aim of the investigation was to decide whether these geographical differences were

phenotypic, were at subspecies or species level, or were in the nature of species-complex differences.

Forms of *Tyrophagus* close to, or identical with, those on cheese were known to infest other plant and animal materials. Members of the group were recorded by Osborn (1893) from mushrooms, by Oudemans (1924a) from cucumbers and melons, and by Van den Bruel (1940) from spinach. They were collected from decaying vegetable material such as damp humus (Oudemans, 1924b) and rotted cineraria stems (Volgin, 1949); from decaying fruits, for example, plums, oranges (Banks, 1906) and apples (Zakhvatkin, 1941); and from stored hay (Michael, 1903), copra (Hirst, 1912), fermenting tobacco (André, 1934), seeds, grain (Zakhvatkin, 1941), meals, nuts and dried fruit (Hughes, 1948). Their association with animal materials was known to be equally widespread. One form was found on the dead larvae of insects (Banks, 1915), others in rodent holes (Zakhvatkin, 1941), in ants' nests (Volgin, 1948) and in birds' nests (Woodroffe, 1953). *Tyrophagus* infestations were recorded from a number of these and similar substances during the present investigation. Again there were differences between populations in body proportions and in relative lengths of dorsal hairs, and again little information was available on which to interpret them. To the first two aims of the present study a third was therefore added: to contribute to the understanding of variation between *Tyrophagus* populations from different host materials.

Material and methods.

Laboratory populations.

Variation within cheese populations was analysed first in the laboratory. A range of cultures was established, each bred up from a nucleus of mites agreeing in the leg, body and dorsal hair characters under consideration. These mites were picked out from bulk samples of infestations occurring in cheese stores in southern and midland districts of England.

Cultures were maintained on a mixture of wheat germ and finely flaked cheese, and held in 2 in. by $\frac{1}{2}$ in. tubes plugged with non-absorbent cotton-wool. Cross infestation was prevented by placing each tube in a 4-in. high, $2\frac{1}{2}$ -in. diameter staining jar, the top of which was closed by a disc of Whatman's no. 1 filter paper sealed to the rim with paraffin wax. When cultures became sufficiently dense they were held at 10°C., in either a constant-temperature room or a refrigerator, at a relative humidity of 80 to 85 per cent., a level comparable to that found in commercial cheese stores. Relative humidity within culture jars was checked with cobalt thiocyanate paper by the method of Solomon (1957). It was found to reach equilibrium with that of the surrounding atmosphere within one to two hours after the jars were set up. Build-up of CO₂ appeared to be negligible, a view supported by the result of tests carried out by Miss E. Reynolds, Pest Infestation Laboratory. With the breeding method used, *Tyrophagus* material required subculturing only at intervals of several months.

Quantitative comparisons.

After breeding the cultures established in the laboratory for twelve months or more, a number were judged to be identical, while others appeared distinctive. Of the latter, culture C, obtained on 9.ii.53 from imported New Zealand cheddar made at Eltham, Taranaki, and stored at Burwell, Cambridgeshire; D, from English farmhouse cheddar, Somerset, collected on 6.xii.50; and F, collected from English cheddar at Reading on 7.iii.51, were selected for detailed comparison by measurement.

All material to be compared was mounted dorsoventrally in gum chloral—Swan's (1936) modification of Berlese's fluid—with no more than two specimens on each slide. Measurements were made at magnifications of from $\times 75$ to $\times 600$,

according to the characters being considered, using a filar eyepiece measuring directly to $\cdot 01$ mm.

The characters analysed, which were combined in pairs as ratios, are illustrated in fig. 1. The *body length* was measured from the posterior margin of the body

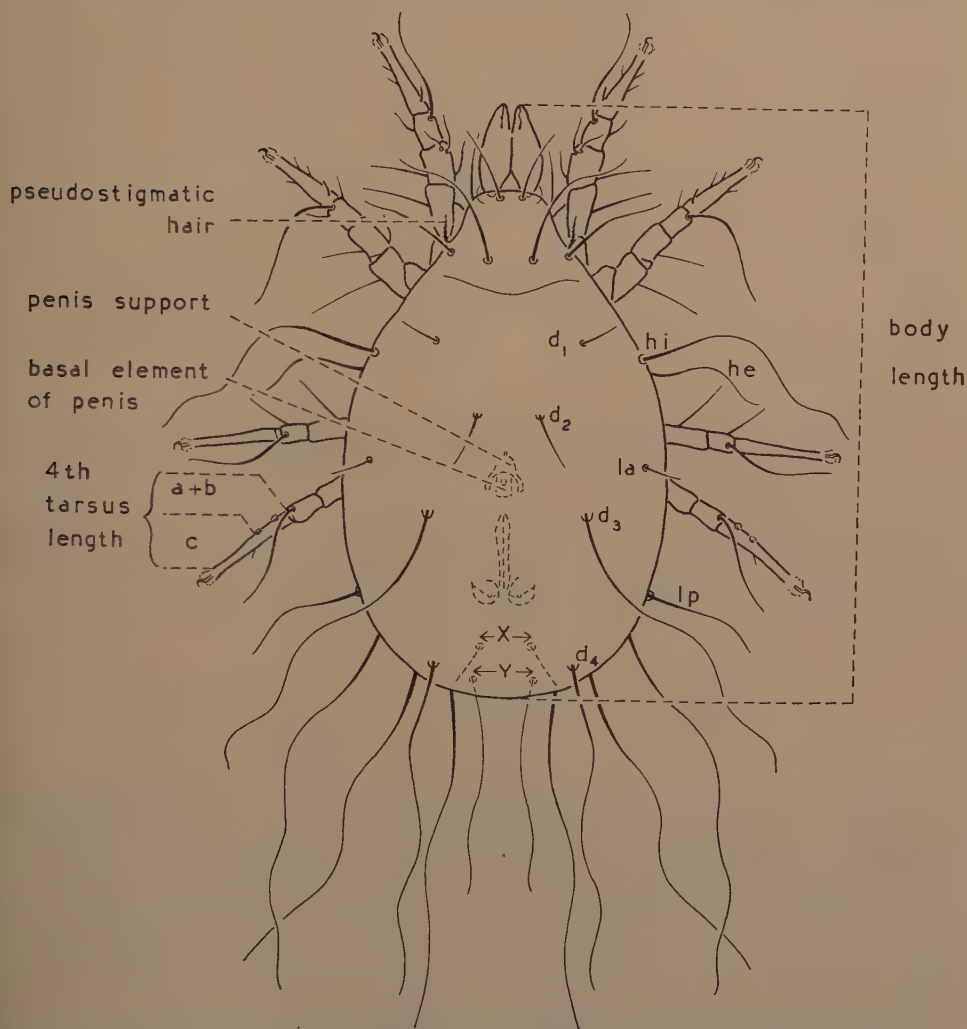


Fig. 1.—Dorsal mount of male of *Tyrophagus longior* showing measurements made for ratios (i), (ii), (iii) and (iv) (pp. 9–14), and some of the structural characters considered (pp. 15, 16).

to the anterior tips of the chelicerae, the chelicera which was further extended being chosen when the two differed in degree of extrusion. The total length of the *fourth tarsus* was taken from its base to the base of the claw, but excluding the latter. The section *a+b* of the fourth tarsus of the male was measured from the proximal end of the tarsus to the distal margin of the second tarsal sucker, and the section *c* from the latter point to the tip of the tarsus, again excluding the claw. Further male characters measured were the distance *X*, between the first pair of postanal hairs, and the distance *Y*, between the third pair. In both

cases these distances were measured from the inner edge of the base of one hair to the inner edge of the base of its fellow.

Measurement of the second dorsal (d_2) and the anterior lateral (la) body hairs was complicated by their flexibility. In living material the hairs were curved and stood out at an angle from the body, while in mounted material they were flattened but seldom lay straight. Moreover they tapered gradually, so that the tips became very fine indeed. Several methods of measurement were considered, such as that of projection and measurement with a map reader, but all proved unsatisfactory because the terminal portions of the hairs could not be traced. Finally, measurements had to be made with the filar eyepiece, using phase contrast to define the tips clearly. The straight distance from base to tip of the hair was measured, and a figure for curvature was added. Since populations were being compared on the basis of the ratio of one hair to another, not on direct measurement, the error introduced by this method of estimating hair lengths depended on the difference in degree of curvature of the components of each ratio. When, as was normally the case, curvature was of a similar order, the significant figures of the ratio were not affected, but an appreciable difference in curvature produced an error of approximately 2 to 5 per cent. Since no more satisfactory technique for measuring hairs could be found, the limitations of this method had to be accepted.

Population comparisons were finally restricted to the measurement of males, since in preliminary studies no significant differences between sexes were recorded in characters common to the two, and since a greater number of measurable characters were available in the males, which were also not subject to the variations in size associated with egg-development.

In a preliminary series of measurements of the ratio of the length of the body to the length of the fourth tarsus in males of the population F, the accuracy of the means obtained with samples of different sizes, as indicated by the standard error, was as follows:—

Number of specimens	Mean	Standard error
10	7.655	± 0.128
25	7.625	± 0.100
50	7.617	± 0.072
100	7.601	± 0.050

For comparisons of the laboratory populations C, D and F, a sample size of 50 specimens was selected as being workable in relation to the time involved in mounting the material, while being large enough to demonstrate the differences between populations with a reasonably high degree of accuracy.

The character ratios measured in each population were plotted as frequencies and analysed by the methods of Simpson & Roe (1939), and of Cazier & Bacon (1949).

The coefficient of variability was calculated from $100 \times \text{standard deviation} / \text{mean}$. The significance of the difference between population means was assessed from values of 't', calculated by standard methods. The percentages of joint nonoverlap of populations were obtained from a table published by Mayr, Linsley & Usinger (1953), and were related to values of the coefficient of difference, C.D., calculated from the formula $M_1 - M_2$.

$$\frac{M_1 - M_2}{S.D._1 + S.D._2}$$

Structural comparisons.

Not only were populations examined for structural characters which had been used to separate species by workers such as Oudemans (1924b), Zakhvatkin (1941), Hughes (1948) and Volgin (1948, 1949), but a search was made for anything additional which might help to clarify relationships.

Some characters, such as the pseudostigmatic hair and the male and female genital openings, could be studied in the dorso-ventral mounts made for quantitative comparisons. Others, in particular the penis, required a special method of preparation. For this a small area of microscope slide was smeared thinly with Berlese's fluid and several live males were placed in it. It was warmed gently so that specimens were relaxed with the legs extended. Each mite was placed on its side, and slight pressure was applied with a fine needle until the penis was everted. Further heating and pressure were required before it became fixed into position by the hardening mountant. The preparation was left uncovered at this stage and was held at 40°C. for 24 hours. It was then possible to add further Berlese's fluid and apply a coverslip without altering the position of specimens (fig. 2).

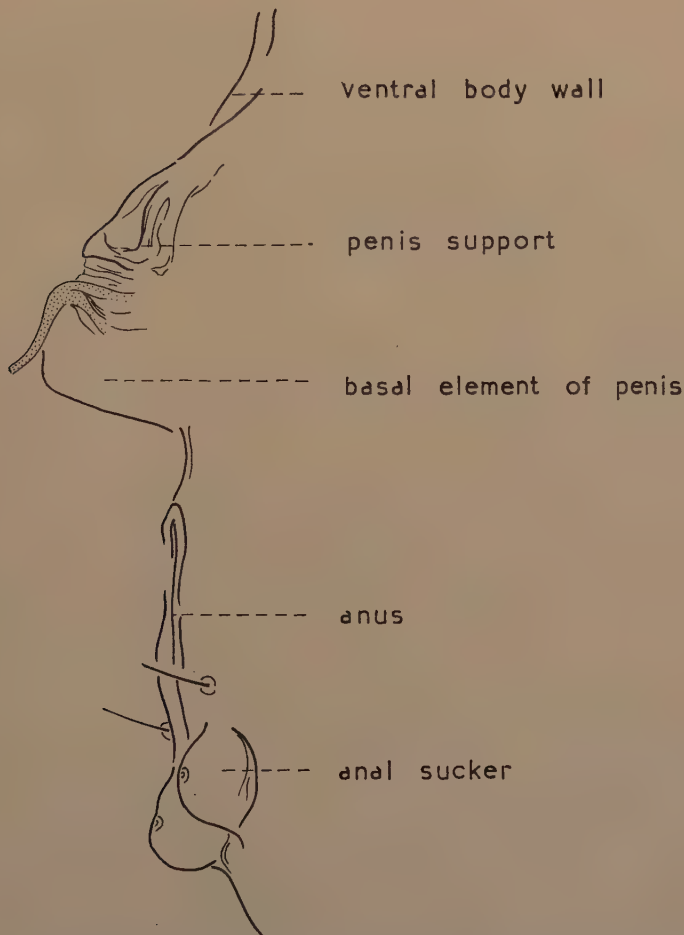


Fig. 2.—Lateral position for mounting male specimens of *Tyrophagus* to show penis characters.

Phase-contrast illumination proved particularly valuable for the study under high magnification of delicate cuticular structures such as the penis, the pseudostigmatic organ at the base of the first pair of legs, and the solenidions and other setae on the first tarsus.



Fig. 3.—Frequencies of $\frac{\text{body length}}{\text{fourth tarsus length}}$ for males of *Tyrophagus* populations C (x), D (□) and F (▲)—interval 0.1.

TABLE I (a).

Variability in $\frac{\text{Body length}}{\text{Fourth tarsus length}}$ for males of populations C, D and F.

Population			Mean	Coefficient of variability
C	5.675	8.66%
D	7.347	4.96%
F	7.441	6.26%

TABLE I (b).

Comparison between males of C, D and F for $\frac{\text{Body length}}{\text{Fourth tarsus length}}$

Comparisons		$t \left[\frac{d}{\sigma_d} \right]$	Joint percentage nonoverlap	Coefficient of difference
C and D	..	***19.15	>96%	ø1.937
C and F	..	***18.32	>96%	ø1.832
D and F	..	1.12	—	0.113

*** Probability level of significance < .001.
ø > conventional subspecific difference level (=1.28) (see Mayr, Linsley & Usinger, 1953, p. 146).

Comparisons between specimens were made under standard illumination by a projection method in which the microscope light beam, passing up through condenser and objective, was deflected at right angles by a prism placed on top of the eyepiece. This beam was turned back on the bench by a large, surface-aluminised mirror mounted at 45°. The base of the mirror support, which was screwed to the bench, contained a 5-inch slot along which the mirror could be moved for the final adjustment of magnification. To guard against distortion and changes in magnification during drawing, the field was projected on to a circle outlined on stiff white mounting card. A series of circles of different sizes were used as magnification standards.

Store and field samples.

When the study was extended to store and field populations of *Tyrophagus* collected in different geographical areas and from various host materials, comparisons were based on small samples of ten mites per sample.

Geographical differences were considered in populations from cheese produced in several Commonwealth and European countries. Some samples were collected after cheese reached United Kingdom stores, but were accepted as having originated overseas on the basis of earlier observations on the course of cheese infestations (Robertson, 1952). Nine samples, three of each of the types C, D and F covering the widest possible geographical range, were selected for quantitative comparison. These were obtained from the following sources: Type C (i) 16.viii.44, Manawatu, New Zealand, (ii) 19.viii.52, Burwell, Cambs.—infesting New Zealand cheese, (iii) 26.iii.54, Gouda, Netherlands; Type D (iv) 19.viii.52, Burwell, Cambs.—infesting New Zealand cheese, (v) 29.v.52, Luton, Beds.—infesting Australian cheese, (vi) 6.xii.50, Wedmore, Somerset—infesting farm-produced English cheese; Type F (vii) 16.viii.44, Manawatu, New Zealand, (viii) 20.x.50, Sutton Bonington, Leics.—infesting English cheese, (ix) 7.iii.51, Reading, Berks.—infesting English cheese.

The following samples were used to demonstrate relationships between *Tyrophagus* populations from different host materials: (x) 16.x.54, Holbeach St. Johns, Lincs.—in grain, (xi) 18.viii.55, Reepham, Norfolk—in hay, (xii) 22.v.46, Waikanae, New Zealand—on Iceland poppy plants, (xiii) 5.iii.51, Wye, Kent—on mushrooms, (xiv) 7.ii.47, Nelson, New Zealand—in soil and grass, (xv) 7.xii.53, Indianapolis, U.S.A.—in fungal culture, (xvi) 18.iii.52, Slough, Bucks.—in insect culture, (xvii) 31.x.51, Lagos, Nigeria—in palm-kernel dust, and (xviii) 14.iii.55, Western Provinces, Ghana—in copra.

Populations (i)–(xviii) were compared quantitatively by Student's 't' test, and the probability levels of significance of the differences were obtained from Simpson & Roe's (1939) table of 't' for small samples.

Structural comparisons were based on the methods of preparation and examination used for laboratory populations, and the same characters were studied.

Results.

Laboratory populations from cheese.

(i) *The ratio of body length to fourth tarsus length.*—(Tables I (a) and I (b); fig. 3.)

All three populations showed a low level of variability for this ratio. C was almost completely separated from the other two populations, but there was no indication of separation between D and F.

(ii) *The ratio of the length of the second dorsal hair, d_2 , to that of the anterior lateral hair, la^* .*—(Tables II (a) and II (b); fig. 4.)

* Terminology of Zakhvatkin (1941).

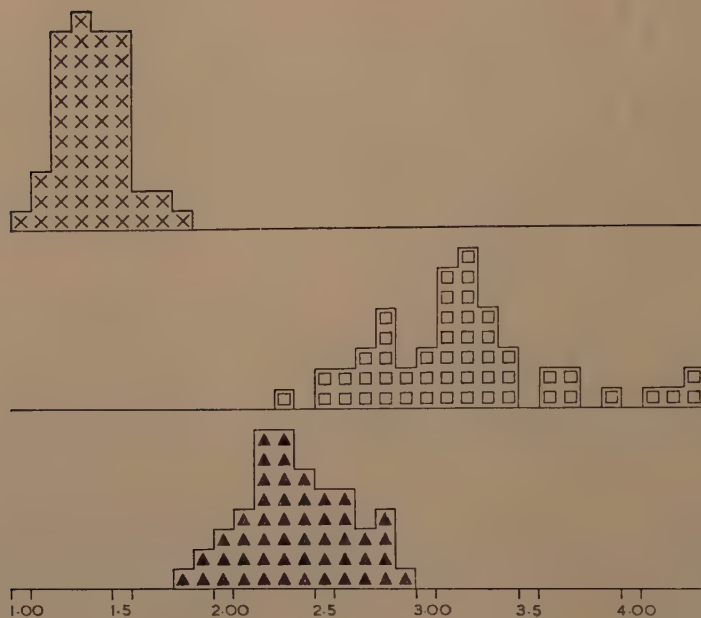


Fig. 4.—Frequencies of the dorsal hair ratio d_2/la for males of populations C, D and F—interval 0.1. Symbols as in fig. 3.

TABLE II (a).

Variability in $\frac{d_2}{la}$ for males of C, D and F.

Population			Mean	Coefficient of variability
C	1.405	11.82%
D	3.221	13.96%
F	2.407	10.89%

TABLE II (b).

Comparison between males of C, D and F for $\frac{d_2}{la}$

Comparisons	$t \left[\frac{d}{\sigma d} \right]$	Joint percentage nonoverlap	Coefficient of difference
C and D ..	***26.78	>96%	02.95
C and F ..	***22.82	>96%	02.34
D and F ..	***11.06	>87%	1.14

There were highly significant differences between means for d_2/la in the three populations, and the frequency distributions for C and D were almost completely separated. The closeness between D and F was again apparent in the lower level of significance of the difference between their means.

Variability was somewhat similar for all three populations, and was considerably higher than for the first character considered. This was to be expected with the difficulty of making measurements and the small size of the components of the ratio. Nevertheless the coefficients recorded were still of a normal magnitude for insect and mite material.

(iii) The ratio of section $a+b$, bearing the suckers of the male fourth tarsus, to the remainder of the tarsus, c .—(Tables III (a) and III (b); fig. 5.)

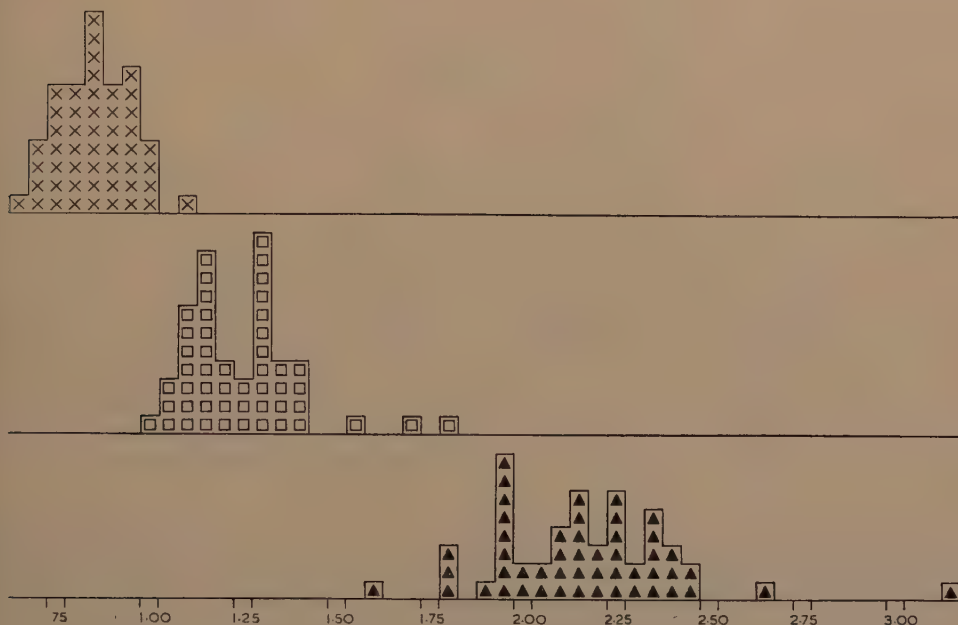


Fig. 5.—Frequencies of the fourth tarsal ratio $\frac{a+b}{c}$ for males of populations

C, D and F—interval 0.05. Symbols as in fig. 3.

Variations in the position of the suckers on the fourth tarsus of the male of *Tyrophagus* had attracted the attention of acarologists for some time before the present study. The Oudemans illustrations at the Leyden Rijksmuseum van Natuurlijke Historie included a plate, published in 1906, showing differences between the forms which Oudemans called *dimidiatus* and *australasiae*, and those illustrated by Berlese as *infestans*, and by Michael as *longior*. Zakhvatkin (1941) also illustrated a gradation of forms of the fourth tarsus in the species he keyed.

The level of variability was similar for the three populations, and very close to that recorded for d_2/la . All comparisons between the means showed that the differences between them were highly significant, but, unlike the previous case, the greatest difference was between C and F, not between C and D.

(iv) The ratio of the distance X, between the first pair of male postanal hairs, to the distance Y, between the third pair.—(Tables IV (a) and IV (b); fig. 6.)

TABLE III (a).

Variability in the tarsal ratio $\frac{a+b}{c}$ for males of C, D and F.

Population			Mean	Coefficient of variability
C	0.873	11.08%
D	1.265	12.25%
F	2.185	11.07%

TABLE III (b).

Comparison between males of C, D and F for $\frac{a+b}{c}$

Comparisons		$t \left[\frac{d}{\sigma_d} \right]$	Joint percentage nonoverlap	Coefficient of difference
C and D	..	***15.19	94%	ø1.555
C and F	..	***35.56	> 96%	ø3.87
D and F	..	***22.66	> 96%	ø2.32

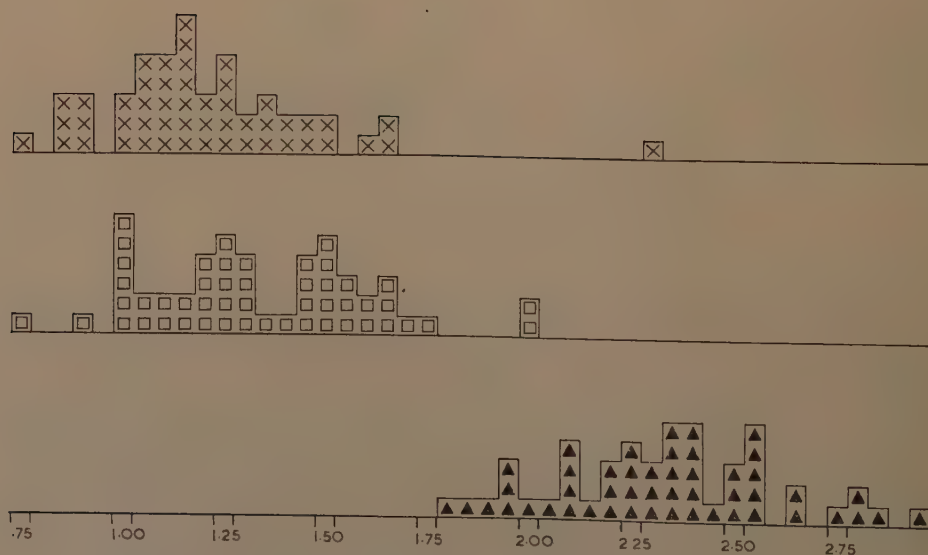


Fig. 6.—Frequencies of the postanal hair separation ratio $\frac{X}{Y}$ for males of populations C, D and F—interval 0.05. Symbols as in fig. 3.

There is some confusion in literature about the naming of postanal hairs, but the two pairs considered here are those which Zakhvatkin (1941) named p_1 and p_3 , respectively, and which Nesbitt (1945) illustrated in his figure 40 as numbers 11 and 13.

TABLE IV (a).

Variability in the ratio of the postanal distances $\frac{X}{Y}$
for males of C, D and F.

Population	Mean	Coefficient of variability
C	1.224	21.31%
D	1.356	17.66%
F	2.355	11.32%

TABLE IV (b).

Comparison between males of C, D and F for $\frac{X}{Y}$

Comparisons	$t \left[\frac{d}{\sigma_d} \right]$	Joint percentage nonoverlap	Coefficient of difference
C and D ..	*2.63	—	0.263
C and F ..	***21.75	> 96%	02.14
D and F ..	***19.63	> 96%	01.97

* Probability level of significance < 0.05.

Measurements of X and Y were made over parts of the soft body cuticle, and were therefore more likely to be reduced or increased by the condition of the body contents, or by variations in mounting, than were the inelastic body hairs or tarsi. This was borne out by large differences in the coefficients of variability recorded for the three populations, and by the excessively high level of two of them. Despite this the character proved a valuable one, since by it F was almost completely separated from both C and D.

A number of other character ratios were considered for the separation of C, D and F, such as length of the first tarsus/length of first genu + tibia; distance between the levels of the 2nd and 3rd dorsal hairs/distance between the levels of the 1st and 2nd dorsal hairs; length of the anus of the male/distance between the anus and genital opening.

Some degree of separation of C from the other two populations was indicated in the first of these ratios, and of D and F, respectively, in the second and third. But they added little to the information brought out by the ratios already considered, which, taken together, demonstrated very significant differences between the three populations. C was separated from D and F by ratio (i), F was separated from C and D by ratio (iv), while all three populations were separated by ratios (ii) and (iii), although with (ii) the greatest difference was between C and D, while with (iii) it was between C and F. Using the 'coefficient of difference' as criterion, each population was confirmed as being at least subspecifically different from the others by one or more of the four ratios.

(v) *Structural comparisons between populations.*—Characters associated with the male and female genital openings, which could be examined without oil immersion with magnifications of about 400 times, were considered first. There were slight differences in the female opening in size, in the degree of separation of the two arms of the inverted 'Y' which marked the opening, and in the extent and pattern of surface sculpturing, but they could not be used as primary points on which to distinguish one population from another. For this the male genital opening was more useful. It is made up of two parts (fig. 1), an anterior curved sclerite which Oudemans called the penis support, and a median structure which is the basal element of the penis. In dorso-ventral view the penis support of C was bell-shaped with the arms turned inwards, and the basal element projecting behind them. In D it was shortened and expanded laterally, but the arms were again turned inwards with the basal element, which was considerably smaller than in C, scarcely extending beyond them. In F the bell shape was completely lost, and the anterior sclerite had the form of an inverted 'V', with the arms straight and the ends turned outwards. The margin of the basal element was expanded into a wide arc, distinct from its straighter outline in C and D.

In C, the anus and male genital opening were separated by only a small space, in D the distance was twice as great, and in F it was at least three times.

When populations C, D and F were compared on the size and shape of the penis, no confusion between the three forms was possible, and they were completely separated by this character.

The form of the pseudostigmatic hair, lying dorsally like an epaulet at the base of the coxa of the first leg (fig. 1) was another character recognised by Oudemans and later workers. That of series C was bent at a characteristic angle and had long pectinations extending along most of its shaft. In D, the pectinations were all comparatively short, while, in F, long pectinations were confined to the enlarged basal half of the organ.

The positions of the setae on the first tarsus (fig. 7), lettered *aa* and *ba* by

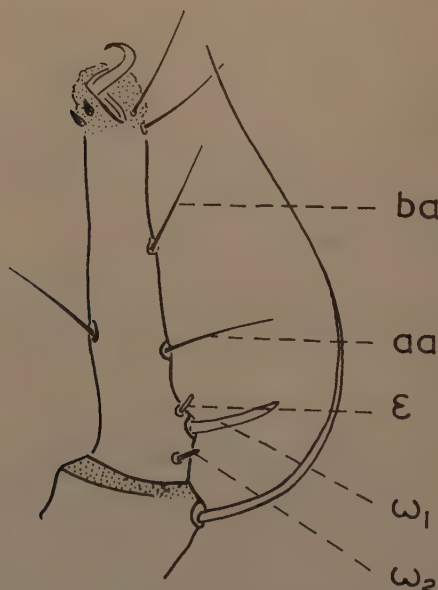


Fig. 7.—Dorso-lateral view of first tarsus, showing solenidions and setae (terminology of Grandjean, 1939).

Grandjean (1939) and called the postdorsal median and dorsal median setae by Nesbitt (1945), differed in the three populations. In C, *aa* lay close to the solenidions ω_1 and ω_2 at the base of the tarsus, with *ba* well separated from them but still in line. In F, *ba* was in the same position but *aa* was further removed from the basal solenidions. In D, *aa* was beside the solenidions, but *ba* had moved from the original line towards the external lateral face of the tarsus. The shape of the solenidion ω_1 , sometimes called the sensory club, was characteristic for each population. In C it was long, slightly curved and tapering towards the tip. In D it was shorter and straight, with its greatest circumference about the centre, while in F the enlargement was at the tip.

(vi) *Status of differences between populations.*—When differences in these minute structures were judged in relation to the measurements made, it became obvious that they could best be explained by assuming that C, D and F were three separate species. From the overlap of ratios it seemed unlikely that the three could be separated by measurement alone, but measurement in conjunction with examination of the form of the pseudostigmatic hair and of the male genital opening proved adequate for separation in all but a few cases.

C was identified as *longior* (Gervais, 1844, pp. 260–266), the species which Oudemans recorded as *dimidiatus* and found in so many habitats (listed by Robertson, 1959); D appeared closest to *palmarum* Oudemans, 1924, which had never been recorded as a storage pest, and had apparently not been recognised since Oudemans found it in the stem of a palm; while F was assigned to *putrescentiae* Schrank, 1781, the type of the genus, which Oudemans collected from humus in 1902 and from a number of other materials in later years.

The fact that cheese was infested not by one species of *Tyrophagus*, the accepted opinion when populations were first sampled, but by at least three, seemed likely to have a bearing on the problem of variation within store populations. Earlier survey samples were re-examined and were in fact found to contain more than one species. Indeed, in all the store material available for study, the most important variations in size and in leg/body relationships could be explained as reflecting differences between species. It had to be decided next how widely the three species so recognised were distributed, and the way in which they were associated in cheese infestations.

Store samples from cheese.

Separation of Tyrophagus species in samples from several countries.—Between 1942 and 1954, 166 collections of mites had been made in cheese stores. From the majority of collections five to ten samples were kept, each of 20 adult mites mounted in Berlese's fluid. Most of this material was collected either in New Zealand or in the United Kingdom, but a little was obtained from other countries. In Table V, records for the 156 collections containing *Tyrophagus* are summarised. It should be pointed out that figures for the numbers of specimens mounted are totals of population samples and include not only specimens of *Tyrophagus*, which were in the majority, but also of several other Acaroid genera.

These records showed *longior*, *palmarum* and *putrescentiae* to be widely distributed as cheese pests in the United Kingdom and Europe, South America, Australia and New Zealand. Taking the records as a whole, it was difficult to obtain a clear picture of differences in geographical distribution between the three forms, but certain points concerning distribution were indicated in the New Zealand collections made between 1940 and 1944 which were of significance because:

(a) Up to the time samples were collected the amount of cheese imported into New Zealand was negligible, so that the cheese mite species present then were unlikely to have been disturbed by the introduction of forms from outside.

(b) Since cheese was stored under atmospheric conditions in the factory curing rooms in which collections were made, the *Tyrophagus* species occurring in this

habitat should follow a more or less natural distribution through the country as a whole.

(c) New Zealand, although small, extended from latitude 35° to latitude 47°, which should perhaps be far enough from north to south to demonstrate obvious species differences in distribution, if any existed.

TABLE V.

Summary of *Tyrophagus* species recorded in mite samples collected from cheese factories and stores.

Locality	No. of collections	Mite specimens mounted	<i>Tyrophagus</i> species identified
New Zealand :			
Manawatu	3	560 *(28)	**C D F
Marlborough	44	8560 (428)	D
Southland	6	1200 (60)	C D
Taranaki	29	5300 (265)	C D F
Wairarapa	3	440 (22)	C D
Australia :			
Queensland	2	160 (8)	C F
Victoria	1	40 (2)	C
South America :			
Chile	1	2 (1)	D
Netherlands :			
Drenthe	2	60 (3)	C
Zuidholland	1	50 (23)	C D
United Kingdom :			
Bedfordshire	2	32 (16)	D
(Australian cheese)			
Berkshire	10	320 (16)	F
Cambridgeshire	31	5420 (271)	C D F
(New Zealand cheese)			
Derbyshire	3	100 (5)	C
Home counties	2	100 (5)	C D F
(Australian and New Zealand cheese)			
Leicestershire	5	360 (18)	C D F
Somerset	7	740 (37)	C D
(U.K., Australian and New Zealand cheese)			
Wiltshire	4	680 (34)	C D
(U.K. and New Zealand cheese)			
Totals :	156	24124 specimens 1242 sample mounts	

* Number of sample mounts. ** C=*longior*, D=*palmarum*, F=*putrescentiae*.

The New Zealand collections did in fact suggest that *Tyrophagus putrescentiae*, *T. palmarum* and *T. longior* differ in geographical distribution (fig. 8). *T. putrescentiae*, for example, was the major species of the three in the province of Taranaki, but passing south it was gradually reduced, and did not appear at all

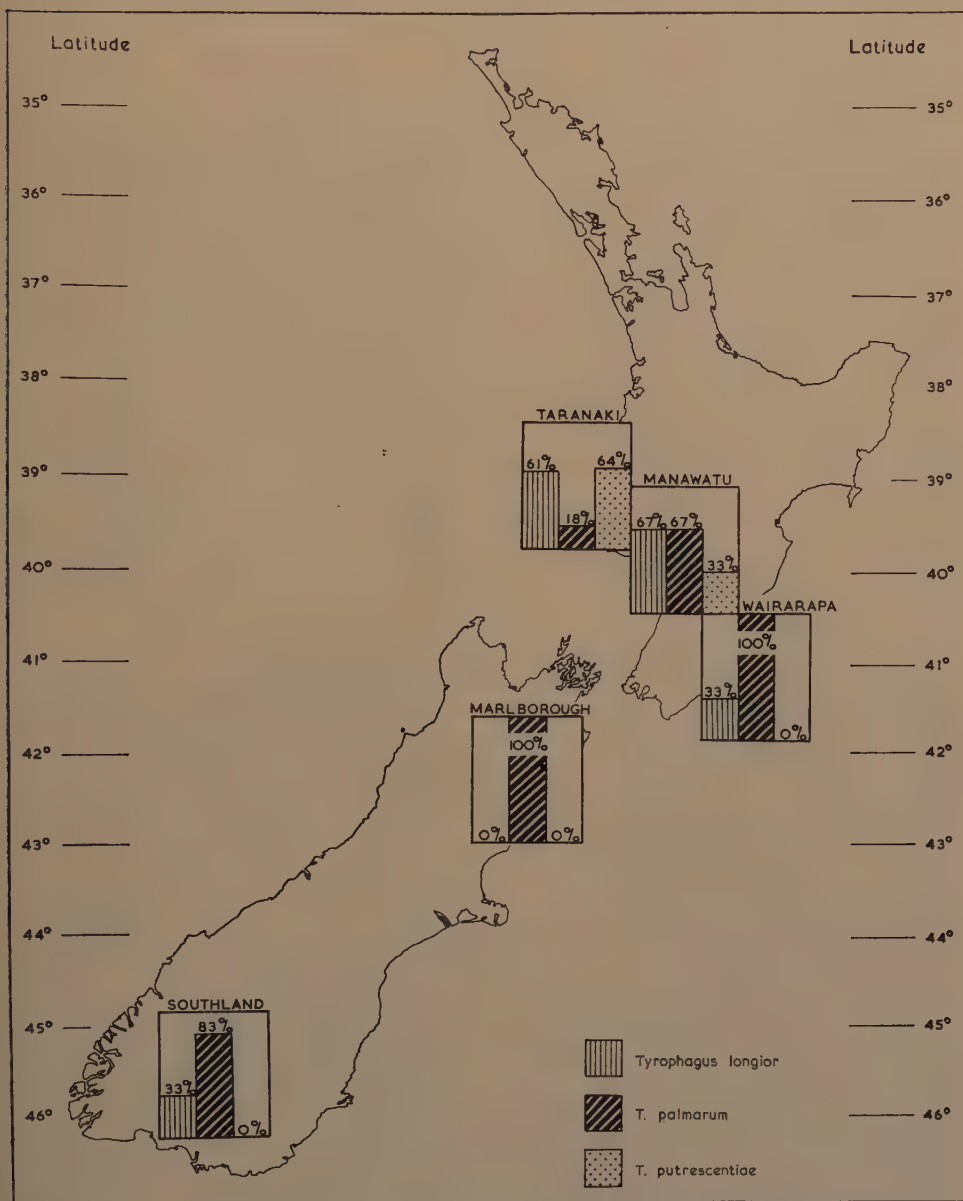


Fig. 8.—The distribution of *T. longior*, *T. palmarum* and *T. putrescentiae* as pests of cheese stored under atmospheric conditions in New Zealand, from an analysis of 56 infestations. For each district histograms represent the percentage of infestations containing each of the three species.

in the South Island. On the other hand, *T. palmarum* was only a minor pest in Taranaki, but increased in importance as *putrescentiae* decreased. It was the major *Tyrophagus* species in the south of the North Island, and in Marlborough at a corresponding latitude in the South Island, although it apparently became slightly less important in the far south (*i.e.*, in Southland). *T. longior* occurred in all the districts in which collections were made, but, except in humid Taranaki, it was of rather minor importance and was generally outnumbered by either *putrescentiae* or *palmarum*, or the two together.

When the world-wide records of Table V were reconsidered in the light of the findings from New Zealand, the general picture appeared to be as follows.

As a cheese pest *T. putrescentiae* is probably a tropical or sub-tropical form which reaches the natural limit of its distribution in New Zealand at about the latitude 39°S. The species was recorded from cheese in Colombia, South America (see p. 524), at a latitude of 10°N.; in southern Queensland at 25° latitude; and in New South Wales at 34° latitude (Robertson, 1959); but it did not appear to attack cheese towards the south of Australia, for example, in Victoria. It was not found as a cheese pest in the cooler northern latitudes of the Netherlands, in which little if any cheese was imported, but in the same latitudes in the United Kingdom, where importations were made from warmer Commonwealth countries, it occurred commonly, although only in stores in which imported cheese was held.

T. palmarum is a temperate form. As well as being the major *Tyrophagus* species attacking cheese in central New Zealand about 40 to 42°S., it was a common cheese species in central Chile, in approximately the same latitudes, while in the northern hemisphere its range extended at least to a latitude of 51° in the Netherlands.

T. longior appears to be a temperate-to-cool climate form, although its basic requirement may be a high atmospheric humidity rather than a low temperature. While it was of rather minor importance in New Zealand between the latitudes of 39 to 47°S., there were indications that it was the major cheese pest species of the genus *Tyrophagus* in the more humid United Kingdom midlands and in the northern Netherlands, between the latitudes of 50 and 52°N.

The interrelationships of longior, palmarum and putrescentiae in cheese-store infestations.—In the collections on which Table V was based, *longior*, *palmarum* and *putrescentiae* occurred either singly, in pairs or all three together. To obtain further information on the association of the three forms in mixed populations, and, in particular, on the way in which environment affects their relative abundance, an experiment was set up at a cheese store at Burwell, Cambridgeshire: The experiment was based on four 80-lb. cheeses made in New Zealand seven months before the commencement of the experiment, and all carrying mixed populations of *longior*, *palmarum* and *putrescentiae*. Two of the cheeses were held in the cool-storage section of the store for a year at a constant temperature of 3.3°C. (=38°F.) and a relative humidity of from 84 to 88 per cent. The remaining two cheeses were transferred for the same period to a section without temperature control, which in winter had weekly temperatures ranging from a mean minimum of 2.9 to a mean maximum of 8.4°C., with corresponding relative humidities of from 68 to 88 per cent.; and which in summer had weekly mean temperatures ranging from 12.2 to 20.2°C., and relative humidities from 54 to 88 per cent. Once a month, ten samples of adult mites were mounted from each population, and a mean figure was determined for the percentage of each *Tyrophagus* species present in the population at that time. The changes in relative abundance of the three species which took place over the twelve-month period, based on these percentages, are illustrated in fig. 9.

In the populations I and II on cheeses stored at atmospheric temperatures and humidities, the three species persisted throughout the year, and at the end of it still made up virtually the whole population. In both cases the proportion of

longior increased during the winter and was reduced in favour of *palmarum* during the summer. In neither case did *putrescentiae* become a dominant species, although it tended to increase in the summer months and to disappear in the winter. In the cool-storage populations III and IV both *palmarum* and *putrescentiae* occurred only in very small numbers, and the winter species *longior* alone built up to significant proportions. But *longior* increased only in the early stages

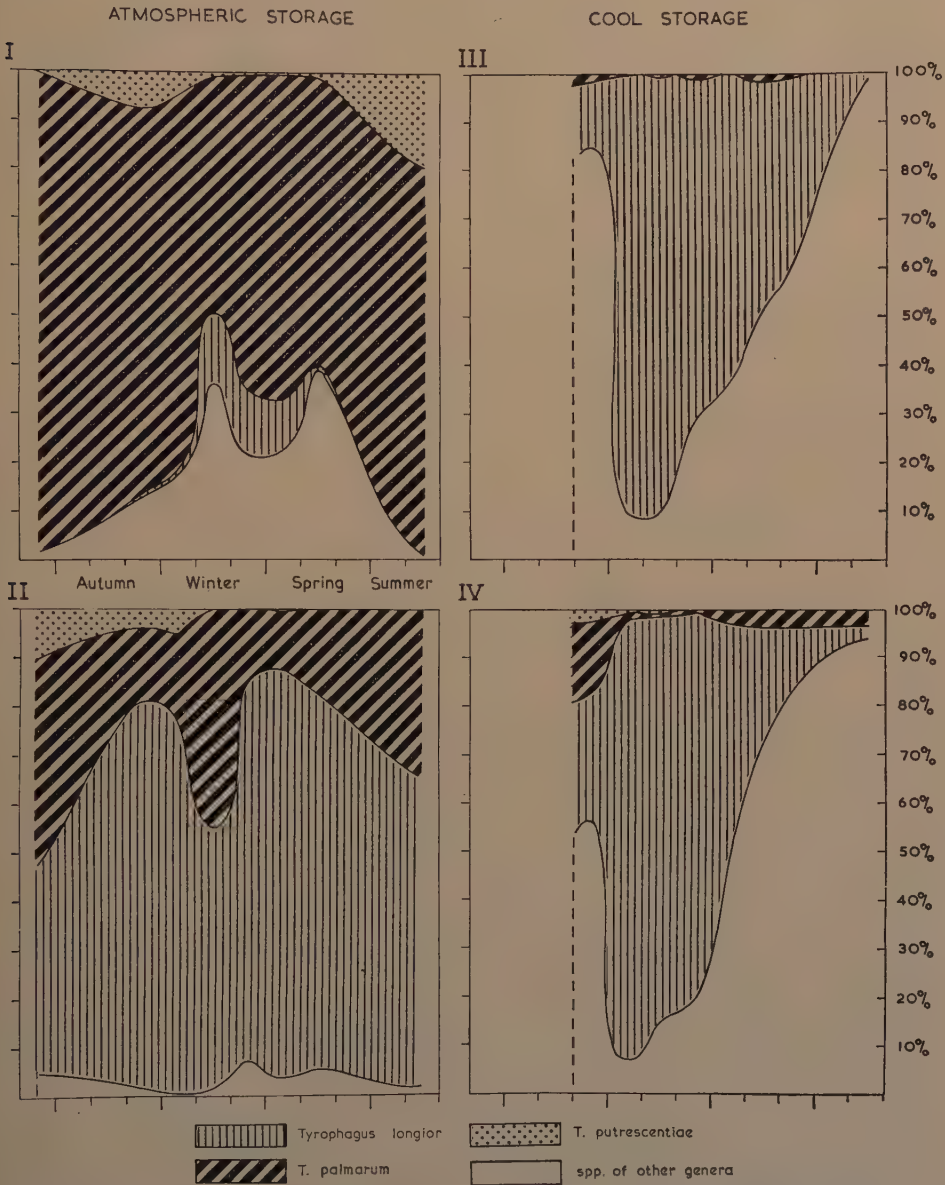


Fig. 9.—Changes in the relative abundance of *T. longior*, *T. palmarum* and *T. putrescentiae* in mixed populations on cheese held for 12 months under atmospheric conditions (populations I & II) and in cool storage (populations III & IV).

of storage and it was then rapidly eliminated by competition from species of other genera.

The experiment supported the view of the temperature characteristics of the three *Tyrophagus* species suggested by their geographical distribution. *T. putrescentiae* appeared to require higher temperatures than those at which the experiment was conducted; *palmarum* was maintained and increased in the intermediate range from 10 to 20°C., even though humidities were reduced to the 55 to 70 per cent. level for a few hours daily; while *longior* was most successful at low temperatures of from 3 to 10°C., particularly when the relative humidity was maintained constantly at the high level of 85 to 90 per cent., as in cool storage. The importance of temperature and humidity in determining the relative abundance of *longior*, *palmarum* and *putrescentiae* was reflected in the different composition of the atmospheric and cool-storage populations. It was seen too in the fluctuating pattern of species relationships in the atmospheric-storage populations, in which

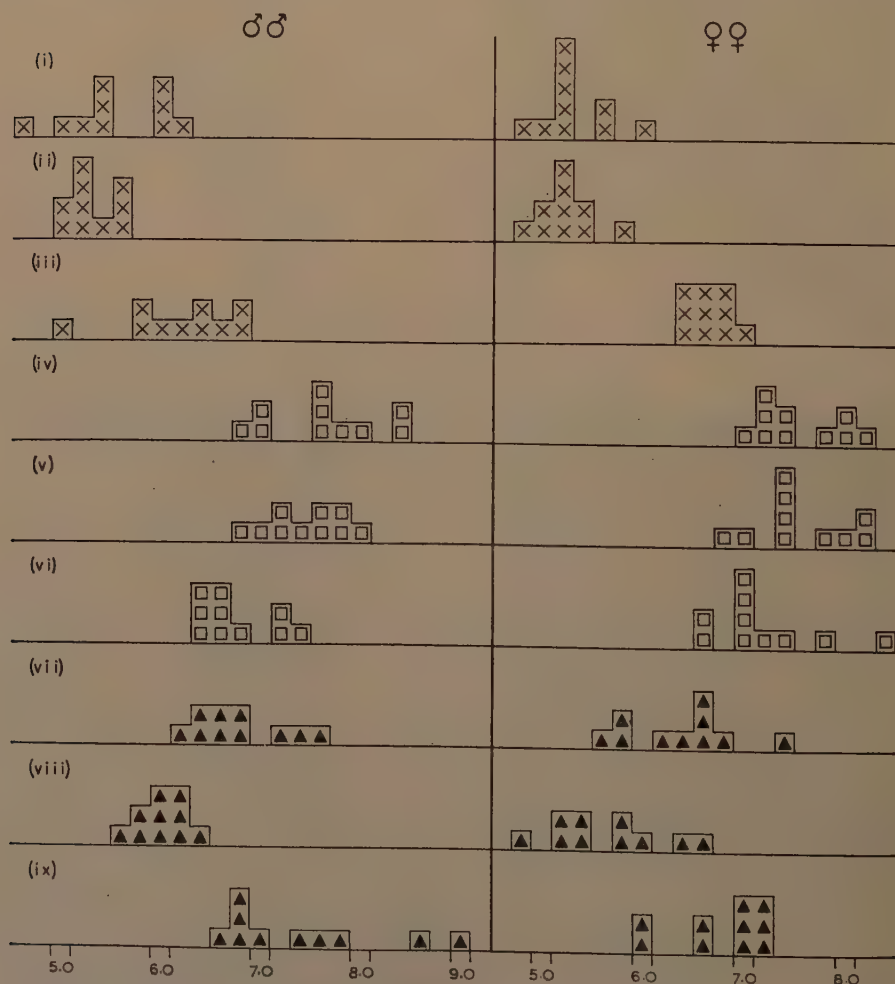


Fig. 10.—Frequencies of body length/fourth tarsus length in small samples of males and females from cheese-store populations of C (=longior), D (=palmarum) and F (=putrescentiae). Symbols as in fig. 3.

the species most favoured by a seasonal change in temperature level increased directly in response to the change.

Intraspecific variation in samples from several countries.—Once *longior*, *palmarum* and *putrescentiae* could be separated with certainty in cheese-store samples, it became possible to examine variation below species level, although only small samples were available for the purpose. The body length/fourth tarsus length (fig. 10) and d_2/la ratios, which had proved satisfactory for the initial separation of species in the laboratory, were compared both between and within species in the nine store samples selected from different geographical areas.

TABLE VI.

Comparisons of body length/fourth tarsus length in samples of males from cheese-store populations of C (= *longior*), D (= *palmarum*) and F (= *putrescentiae*).

Population means	Comparisons	Differences between means	t	P
(i) 5.61	C { (i) & (ii)	.23	1.40	—
(ii) 5.38		.66	2.81	*
(iii) 6.27		.89	4.53	***
(iv) 7.72	D { (iv) & (v)	.18	.84	—
(v) 7.54		.89	4.17	***
(vi) 6.83		.71	4.08	***
(vii) 6.87	F { (vii) & (viii)	.79	4.86	***
(viii) 6.08		.66	2.27	*
(ix) 7.53		1.45	5.46	***
	C & D (ii) & (iv)	2.34	12.73	***
	C & D (iii) & (vi)	.56	2.59	*
	C & F (ii) & (ix)	2.15	8.22	***
	C & F (iii) & (viii)	.19	.97	—

P=Probability level of significance: * < .05; *** < .001.

All means fell within ± 3 standard deviations of the means for the corresponding species calculated in Table I from measurements of laboratory populations. But the separation of *longior* from the other two species, which was obvious in the homogeneous laboratory samples, was obscured in the field samples because some means, such as those of (iii) (*longior*) and (viii) (*putrescentiae*), lay on the overlapping part of the ranges of the two species.

Within species there were some highly significant differences in the body length/fourth tarsus length ratio, and these appeared to be related to the geographical origin of the samples. Of the three populations of *longior* (=C), the significant differences were between (iii) from the Netherlands and both populations from New Zealand cheese, not between the two latter. There was no significant difference between (iv) and (v), the two *palmarum* populations from New Zealand and Australian cheese, respectively, but both differed significantly from (vi), on English farm cheese. Again, (vii) on New Zealand cheese differed significantly from (viii) and (ix), the two *putrescentiae* populations from English cheese. The significant difference between (viii) and (ix) was the only one recorded between populations of similar geographical origin, and might be explained on the assumption that the English cheese from which (ix) was collected had become infested from New Zealand cheese which was being held in the same store.

In contrast to the variability of the 'body:fourth tarsus' relationship, for d_2/la no intraspecific differences reached even the five per cent. level of significance, although all differences between species were highly significant.

Store and field samples from a range of plant and animal materials.

(i) *Separation of species in samples from several materials.*—In comparing infestations of *Tyrophagus* on cheese with those from other materials, use was made of the d_2/la ratio for the initial separation of species.

TABLE VII.

Comparisons of d_2/la in samples of males from store populations of C (= *longior*), D (= *palmarum*) and F (= *putrescentiae*).

Population means	Comparisons	Differences between means	t	P
(i) 1.38	C { (i) & (ii)	.11	1.50	—
(ii) 1.49		.03	.41	—
(iii) 1.41		.08	.88	—
(iv) 3.79	D { (ii) & (iii)	.28	1.73	—
(v) 3.51		.38	2.06	—
(vi) 3.41		.10	.45	—
(vii) 2.43	F { (iv) & (v)	.03	.22	—
(viii) 2.40		.05	.32	—
(ix) 2.38		.02	.17	—
	C & D (vii) & (viii)	2.41	27.65	***
	C & D (i) & (iv)	1.92	10.48	***
	C & F (ii) & (vi)	1.05	8.31	***
	C & F (i) & (vii)	.89	7.36	***
	D & F (ii) & (ix)	1.41	10.87	***
	D & F (iv) & (ix)	.98	4.67	***
	D & F (vi) & (vii)			

TABLE VIII (a).

The dorsal hair ratio d_2/la in male populations of *Tyrophagus* identical with, or close to, *longior*.

Collection	Host material	Population mean
(ii)	cheese	1.49
(x)	grain	1.35
(xi)	hay	1.40
(xii)	Iceland poppy plants	1.03
(xiii)	mushrooms	1.10
(xiv)	soil and grass	1.05

Comparisons	Differences	t	P
(ii) & (x)	.14	} greatest value	—
(ii) & (xi)	.09		—
(x) & (xi)	.05		—
(xii) & (xiii)	.07	} greatest value	—
(xii) & (xiv)	.02		—
(xiii) & (xiv)	.05		—
(ii) & (xii)	.46	6.68	***
(ii) & (xiii)	.39	4.39	***
(ii) & (xiv)	.44	5.08	***

Here results were as clear-cut as in Table VII separating the three cheese species of *Tyrophagus*. Samples (x) and (xi) were apparently identical with (ii), the *longior* from cheese, but a second species was involved in samples (xii), (xiii) and (xiv). In these samples the form of the genital opening in the male was similar to that of *longior*, but the ends of the arms of the penis support were further expanded, and its basal element was wider and curved. The penis itself was bent only once, instead of twice as in *longior*, and the terminal section was spatulate, not slender and tapering. The clubbed tip of the solenidion ω_1 was like that of *putrescentiae*, but further enlarged, and the pectinations of the pseudostigmatic hair were shorter than in *longior*. The most striking characteristic of the species was its short and fine d_1 , d_2 and *la* hairs.

TABLE VIII (b).

The ratio d_2/la in male populations of *Tyrophagus* identical with, or close to, *putrescentiae*.

Collection	Host material	Population mean
(viii)	cheese	2.40
(xv)	fungal culture	2.47
(xvi)	insect culture	2.24
(xvii)	palm-kernel dust	1.07
(xviii)	copra	1.35

Comparisons	Differences	t	P
(viii) & (xv)	.07	} greatest value 3.21	—
(viii) & (xvi)	.16		—
(xv) & (xvi)	.23		**
(viii) & (xvii)	1.33	21.87	***
(viii) & (xviii)	1.05	12.22	***
(xvii) & (xviii)	.28	5.43	***

It was found on seedling plants, and occurred indoors only in mushroom houses, where the association appeared to be with the living mushroom tissue. Of the forms of *Tyrophagus* considered so far it was the only one which could be accepted as entirely a field species, since it apparently occurred in relation to growing plant tissue and had never been implicated as a stored-product pest. It is the species which Oudemans (1926) described when he thought he had found the *infestans* of Berlese, the species called *humerosus* by Zakhvatkin (1941), and the one which has recently been described by the writer as new under the name *oudemansi* (Robertson, 1959).

Populations of *T. palmarum* were not recorded from other materials to the same extent as were members of the *longior* and *putrescentiae* complexes, but samples were obtained from ham, hay and sultanas. The d_2/la ratios were very close to those recorded for cheese populations, and none of the 't' values calculated reached even the 5 per cent. level of significance.

No structural differences could be found separating samples (viii), (xv) and (xvi), but between the two latter there was a difference in the d_2/la ratio reaching the 1 per cent. level of significance. This was of particular interest since it was the only case in which a difference in d_2/la reached a significant level without other specific structural differences being found.

Differences between the cheese populations of *putrescentiae* and samples from

TABLE IX (a).

Sum of the dorsal hair lengths d_2+la in male populations of *T. longior* from various host materials.

Collection	Host material	Population mean (μ)	
(ii)	cheese	142.91	
(x)	grain	83.26	
(xi)	hay	87.36	

Comparisons	Differences (μ)	t	P
(ii) & (x)	59.65	6.82	***
(ii) & (xi)	55.55	5.47	***
(x) & (xi)	4.10	0.63	—

TABLE IX (b).

Sum of the dorsal hair lengths d_2+la in male populations of *T. oudemansi* from various host materials.

Collection	Host material	Population mean (μ)	
(xii)	Iceland poppy plants	53.69	
(xiii)	mushrooms	36.14	
(xiv)	soil and grass	45.13	

Comparisons	Differences (μ)	t	P
(xii) & (xiii)	17.55	5.86	***
(xii) & (xiv)	8.56	3.06	**
(xiii) & (xiv)	8.99	3.74	**

TABLE IX (c).

Sum of the dorsal hair lengths d_2+la in male populations of *T. putrescentiae* from various host materials.

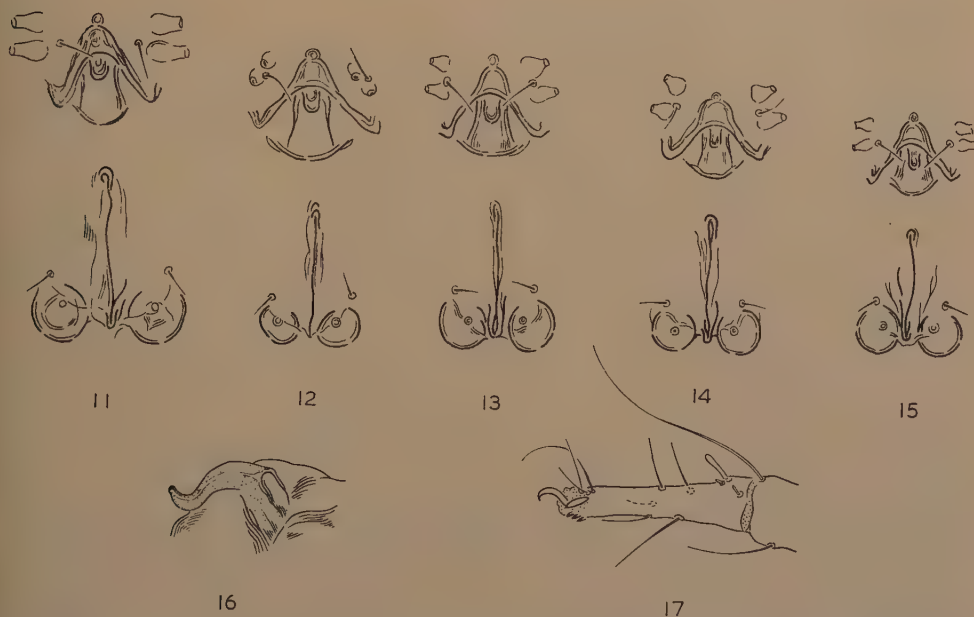
Collection	Host material	Population mean (μ)	
(viii)	cheese	150.35	
(xv)	fungal culture	137.27	
(xvi)	insect culture	79.42	

Comparisons	Differences (μ)	t	P
(viii) & (xv)	13.08	2.16	*
(viii) & (xvi)	70.93	16.13	***
(xv) & (xvi)	57.85	11.35	***

palm-kernel dust and copra collected in Africa were at species level, and formed the basis for two new species, *tropicus* and *brevicrinatus* (Robertson, 1959).

(ii) *Intraspecific variation in samples from several materials*.—Within each of the groups (ii)–(xi), (xii)–(xiv) in Table VIII (a), and (viii)–(xvi) in Table VIII (b), populations were thought to be specifically identical, but were re-examined for signs of genetical divergence below species level. Although, with only one exception, the ratio of d_2 to la was remarkably constant within each group, there were obvious differences in hair lengths between populations. A measure of the difference was obtained from the sum of $d_2 + la$, and populations were compared on this character.

Intraspecific variations in the length of $d_2 + la$ in populations from different host materials were comparable to variations recorded in the body length/fourth tarsus length ratio in store populations on cheese (cf. Table VI). In the present



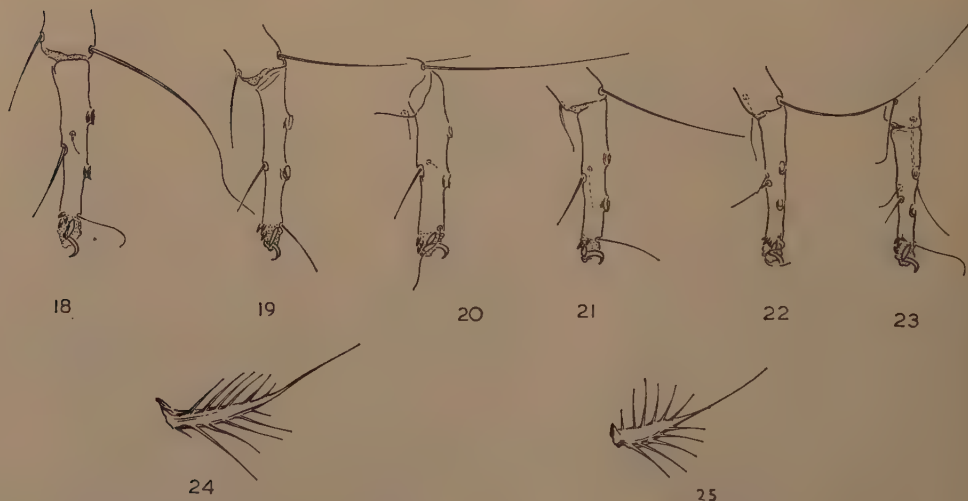
Figs. 11-17.—*T. putrescentiae*. (11-15) Variation in relationships of male genital opening, anus and anal suckers in specimens from various habitats in U.S.A. and Mexico. (16) Penis of a specimen from Mexico. (17) First tarsus of a specimen from Mexico.

case, however, both geographical and habitat differences were involved, and could not be separated. In the *longior* complex, for example, the cheese population, which was quite distinct from the others on hay and grain, differed in its country of origin as well as in its habitat. In *oudemansi*, all three populations were easily separable, although here two of them differed only in their host materials. The highly significant differences recorded in comparing *putrescentiae* population (xvi) from an insect culture with (viii) from English cheese, and also with (xv) from an American fungal culture, appeared to depend on habitat alone in the first case, but in the second must have involved geographical as well as habitat factors.

Intraspecific variations which could not be analysed satisfactorily by measurement were indicated in a collection of 42 slides loaned by Dr. E. W. Baker, United States National Museum, Washington. This material had been collected

from many habitats, and all belonged to the species known as *lintneri* in the United States, and synonymised with *putrescentiae* by the present writer (Robertson, 1959). The specimens illustrated in figs. 11 to 25 were obtained from cheese (Colombia, 9.v.47; figs. 11, 20, 21), bee pollen (Michigan, 1952; fig. 12), a drug-store (Norfolk, Virginia, 16.v.45; figs. 13, 19), an insect culture (Wilmington, Del., 30.vi.52; figs. 14, 22), kitchen shelves (Arlington, Virginia, 1.vi.45; figs. 15, 23), Irish potato (Mexico, 7.v.48; figs. 16, 17, 18), tomato (Mexico, 18.i.50; fig. 24), stored rice (Crowley, La., 7.iii.45; fig. 25).

Size variations were obvious, but in addition there were habitat-related differences in the form of the anal suckers in relation to the parts of the male genital opening (figs. 11–15), the degree of stoutness of the legs and the extent to which they tapered towards the tips (figs. 18–23).



Figs. 18–25.—*T. putrescentiae*. (18–23) Variation in size, shape and sucker position in male fourth tarsus in specimens from various habitats in U.S.A. and Mexico. (24–25) Pseudostigmatic hair in a specimen from U.S.A. and Mexico, respectively.

Discussion.

Baker (1954) considered that *Tyrophagus* contained the most important food-infesting mites in the U.S.A. Material examined in the course of the present investigation is sufficient to give some further indication of the economic importance of the genus, since it has been drawn from Australia, New Zealand and other Pacific islands, parts of Africa, the United Kingdom and Europe. It suggests that species of *Tyrophagus* are also the most important mites infesting stored products in Australia and New Zealand, that in the United Kingdom they probably share first place with the grain mite, *Acarus siro* L. (= *Tyroglyphus farinae* (L.)), which is particularly favoured there by conditions of high humidity in storage, and that they may well prove to be very common in the tropics.

Zakhvatkin (1941) pointed to the difficulty of separating species of *Tyrophagus*, and this is emphasised by the present study. Remarkable resemblances between species have been demonstrated in size and general body form, in the smooth, semi-transparent, whitish cuticle, in the number, position and form of body hairs, in the shape and chitination of the apodemes, and in the structure of the mouth-parts, down to their most minute accessory spines. Preliminary separation of

species can sometimes be made on the relative lengths of certain hairs, for example, the second dorsal, d_2 , and the anterior lateral, la , and the positions of the suckers on the male fourth tarsus. But identity has to be confirmed on such difficult characters as the form of the pseudostigmatic hair, and in particular on the shape of the male genital opening and of the penis, for which special preparations are necessary. Even so, in a population containing more than one *Tyrophagus* species, the identity of at least some female specimens is likely to remain doubtful.

Oudemans, in the period from 1906 to 1924, drew attention to the difficulty of deciding the status of forms of *Tyrophagus* occurring in different countries. But his ideas were not followed up by later workers, so that it has remained for the present study to compare populations differing in origin and, based on the results, to attempt to define the limits of geographical distribution of the species of *Tyrophagus*. So far, six widely distributed pest species have been distinguished, of which *longior*, *palmarum* and *putrescentiae* are the most common. As a group, the three are more or less world-wide in distribution, but considered separately the first of them is a temperate-to-cool form, the second appears to favour temperate areas while the third is found in tropical or sub-tropical parts of the world. In effect, their geographical ranges overlap so consistently that it is difficult to separate them satisfactorily on any characteristic of distribution. Of the remaining three pest species, only *oudemansi* has the same widespread distribution as the first three, although like *palmarum* it tends to occur particularly in temperate regions. It appears to be further restricted by requiring in its environment some factor or factors associated with living plant tissue and soil, and up to the present is known only from habitats of this type. The distribution of the remaining two species, *tropicus* and *brevicrinatus*, is apparently confined to the tropics. The former has been recorded as occurring widely in eastern and western Africa and in the Pacific, but so far the latter has not been found anywhere but in Ghana.

In arriving at a general view of the similarities and underlying differences in geographical distribution of pest species of *Tyrophagus*, it is recognised that the species must also differ, at least in some respect, in the ecological niches which they occupy. For, as Odum & Odum (1959) have affirmed, according to basic ecological principles no two species can occupy exactly the same ecological niche and still be different, although, especially if they are closely related, they may have almost the same niche requirements. Certainly the association of pest species of *Tyrophagus* in mixed populations, which the present investigation has shown to be one of their most striking characteristics, suggests that they must have closely similar niche requirements. In the case of *longior*, *palmarum* and *putrescentiae*, for example, all three live on cheese, all three flourish at high atmospheric humidities—within the 80 to 95 per cent. relative humidity range, according to the habitats in which infestations are normally found—and all three are able to live at temperatures of from 5 to 25°C. But, as with their geographical distribution, the three species are differentiated ecologically by the zones of temperature which favour them most.

When *longior*, *palmarum* and *putrescentiae* are associated in mixed populations, it is assumed that the fate of each species in the complex will depend on the interaction of its intrinsic rate of increase (as defined by Birch, 1948)—that is, its biotic potential—with extrinsic factors of its environment, including physical factors such as temperature and humidity, and biological factors such as the presence of other species. It is already clear that the interaction can be changed by physical factors, and there are also indications that it may be changed by competition from other species. For mite populations on stored cheese tend rather to build up in a mass, perhaps several inches deep, on the surface of one cheese than to migrate to another cheese. Competition in this mass must be very strong indeed for access to the restricted food and space afforded by the cheese surface. Under constant, cool-storage conditions a clear-cut result of competition

can be seen in the complete elimination of *Tyrophagus longior* by species of other genera, but under the varying conditions of atmospheric storage the course of competition is reversed from time to time, depending on the species which are associated.

The present study has produced at least limited answers to each of the specific questions formulated in the introduction. The first of these, concerning marked variation within *Tyrophagus* populations, is explained as indicating the presence of more than one species. As it was suspected at the commencement, the morphological characteristics of these populations can be altered by environment. However, the alteration is not a direct phenotypic effect on body size, leg and hair lengths, but rather a selective effect, in which the one species most favoured by the environment increases at the expense of the species with which it is associated.

The answer to the second query concerning variation between populations originating from different geographical areas suggests a population difference at one of three levels, namely, (i) at the racial level, as in the case of the *longior* on cheese in New Zealand which differs significantly in some morphological details from that occurring in the Netherlands; or (ii) at the species level, where a product is attacked by one species in a certain area and by a second species elsewhere, as cheese is infested by *putrescentiae* in Queensland and by *palmarum* in Marlborough, N.Z.; or (iii) at the species-complex level, as in the occurrence of the *longior-putrescentiae* complex on cheese in Taranaki, N.Z., in contrast to the *longior-palmarum* complex in Southland.

The third query concerning variation between populations infesting different host materials is explained on the basis of either the recognition within a single species of statistically separable habitat races, such as the form of *longior* attacking cheese as compared with the forms living in hay and grain, or the isolation of distinctly habitat-related species in addition to the well-known forms, for example, in the case of the separation of *oudemansi*, living on plant materials, from the closely similar, but polyphagous, *longior*.

Some general implications may also be drawn from the results of the present study. The complexity of the problem of determining the origins of stored-product mite infestations is emphasised, for the results lay particular stress on habitat differences between populations, that is, both morphological and habitat-preference differences. These differences suggest that forms from the field will tend to die out under storage conditions through replacement by others better adapted, and probably permanently related, to storage, from which they may differ specifically or at least racially.

Again, the present results suggest that a number of errors in ecological data published on *Tyrophagus* must have arisen from difficulty in identifying closely similar species, together with failure to recognise the mixed nature of infestations in which they are associated and the dynamic changes which may occur in their relationships. For instance, in the case of the rather dissimilar accounts of *longior* described from cheese in the United Kingdom by Eales (1918), in New Zealand by the writer (1946), in Belgium by Bollaerts & Breny (1951) and in Argentina by Mayer & Hack (1953), only the first of these appears to concern the species now known as *longior*, while the second refers to *palmarum* as the major species and the third and fourth to *putrescentiae* (Robertson, 1959). The most recent case of confusion of this type occurs in the work of Griffiths (1960), who states that *palmarum* and *pernicius*, two species separated completely by male genital characters (Robertson, 1959), are identical, and who considers that *palmarum* is a field species which only accidentally becomes associated with stored foods. The *palmarum* records of the present work suggest that the latter view is incorrect and may perhaps be based on a misidentification.

Finally, the findings of the present investigation concerning the extensive overlapping of the geographical and ecological ranges of pest species of *Tyrophagus* go

far towards explaining the virtually world-wide distribution of the genus, the great variety of materials which are attacked, and the widely varied physical conditions under which infestations are observed to develop.

Summary.

The difficulty of deciding the systematic status of variations within and between populations of *Tyrophagus* is considered in relation to the wide distribution of the genus and the many materials it infests.

Variation within populations of what was thought to be one species infesting cheese is explained by the isolation of three closely similar forms which it is difficult, if not impossible, to separate by measurement alone. Frequency distributions overlap slightly for four linear measurement ratios on which separation is attempted, but differences between them are emphasised by differences in minute structural characters and they are therefore accepted as distinct species, identified as *longior* (Gervais, 1844), *palmarum* Oudemans, 1924, and *putrescentiae* (Schränk, 1781).

Variation between populations attacking plant materials and other stored products in addition to cheese is accounted for to some extent by the recognition of three further *Tyrophagus* species, *oudemansi* Robertson, 1959, *brevicrinatus* Robertson, 1959, and *tropicus* Robertson, 1959.

Within the species recognised, significant differences are recorded in dorsal hair characters and body proportions between populations on cheese produced in different geographical areas, and also between cheese populations and those infesting other materials, and these differences are thought to be at a racial level.

T. longior, *T. palmarum* and *T. putrescentiae* are found to be distributed around the world, but as cheese pests they occupy somewhat different, although overlapping, geographical zones, since *longior* is a temperate-to-cool form, *palmarum* a temperate form and *putrescentiae* a subtropical or tropical form. The three are also found to occupy different, but overlapping ecological zones, making possible their association in mixed populations.

Confusion in ecological data previously published on *Tyrophagus* is thought to be attributable in part to similarities in the morphology, geographical distribution and ecology of its species, and to their close association.

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THE SUSCEPTIBILITY OF TSETSE FLIES TO TOPICAL APPLICATIONS OF INSECTICIDES. I.—YOUNG ADULTS OF *GLOSSINA MORSITANS* WESTW. AND CHLORINATED HYDROCARBONS.

By G. F. BURNETT

Tropical Pesticides Research Institute, Arusha, Tanganyika.

Tsetse flies are among the few insect pests commonly attacked with space sprays, for example, aerial and ground-fog applications and flit-gun sprays in de-flying chambers. Topical applications, therefore, are unusually relevant to problems arising in control work. The Tropical Pesticides Research Institute (formerly Colonial Pesticides Research Unit) has made a number of large-scale trials using solutions of insecticide as aerosols to control tsetse fly in the field. Because tsetse flies, especially the savannah species that were concerned, are unsatisfactory for laboratory work, only a minimum of this was done, but the results were not inconsistent with those of field trials (*e.g.*, Hocking & others, 1953; Hocking, Burnett & Sell, 1954; Hocking & Yeo, 1956). With the advent of new insecticides, a new series of field trials was started (Foster, White & Yeo, 1961; Burnett & others, 1961), which gave some surprising results. More laboratory work was initiated in late 1959 to explain these and was facilitated by the availability of a regular supply of field-collected pupae of *Glossina morsitans* Westw., and by the advent of new instruments for the topical application of small quantities of solutions of insecticide. Throughout, the intention of the laboratory work has been to account for the field results and assist in designing new aerial-application experiments.

Methods.

Chlorinated-hydrocarbon insecticides have been applied as solutions in lighting kerosene to the dorsum of the fly's thorax, using the type of microburette due to Kerr (1954). Solvents used in the field have been a mixture of power kerosene and an involatile oil, such as Shell diesolene, Shell furnace oil, a special Shell high-aromatic solvent, or the involatile oil of unknown composition in which the γ BHC concentrate known as Hexastan is supplied by the Standard Disinfectant Co., London. When kerosene is used, most of it volatilises in a few seconds (Yeo & Thompson, 1954) and the insect picks up a saturated or a super-saturated drop. It was found that the smallest drop of these involatile solvents that could be accurately applied was about 0.02 microlitres (μ l.), some hundred times the size of the droplets used in the field. This amount was lethal to *G. morsitans* and the solvents were, in any case, difficult to use in the microburette. For these reasons, lighting kerosene has been used for all applications except in a trial with the involatile Shell Risella 117 (see below). Tests showed that drops of 0.02 μ l. of either of these solvents gave no higher mortalities than those suffered by untreated flies in the same period.

A constant volume of solution was used, 0.0216 μ l., and the concentration was varied logarithmically. The results are given as microgrammes (μ g.) per fly. Because the mean weight of the female tsetse exceeds that of the male and both vary with the season, samples of flies were weighed at intervals immediately before treatment; if desired, the results can thus be expressed as weight per mg. of insect. DDT, BHC and dieldrin were used as pure recrystallised p,p' isomer, γ isomer,

and HEOD, respectively. The new Shell insecticide Telodrin (1,3,4,5,6,7,8,8-octachloro-3a,4,7,7a-tetrahydro-4,7-methanophthalan), formerly known as WL 1650, which is a member of the dieldrin series, was used as the 'pure technical' ($97 \pm 1\%$) material, endrin as 'pure technical' and aldrin as recrystallised technical material.

The tsetse material used consisted of adults of *G. morsitans* that emerged in the laboratory from pupae collected near Singida, central Tanganyika, and sent by the weekly bus to Arusha, a distance of about 220 miles. The pupae were kept in an insectary room with temperature uncontrolled except that it was raised to 27°C. for a few hours each day, when the adult flies were fed. Newly emerged flies were kept individually in 3×1 in. glass tubes in incubators at 26°C. and 70–75 per cent. R.H. until needed. They were offered sheep the day after eclosion and daily until they fed. All flies were used one day after their first feed, when they were 2–5 days old. This procedure secured flies of as constant a physiological state as possible with sufficient food reserves to last three days. Insecticide was applied at room temperature to flies anaesthetised with carbon dioxide. These were at once released into their tubes and confined without further feeding in a constant-temperature room at 26°C. in a cabinet over water, in which the relative humidity was 75–80 per cent. Mortalities were observed at 24, 48 and 72 hours after treatment.

On each day only a few flies were available; even with 500 pupae supplied per week there were rarely more than 30 adults of either sex available for test. Each day the flies available were divided as equally as possible between each insecticide concentration and the control until a minimum of 30 had been exposed at each concentration, when the results obtained over a period of several weeks were combined. There was considerable day-to-day variation in mortalities but in this way significant regression lines were obtained which are unexpectedly good taking into consideration the variation in body weight of the flies (Tables II, III). It was impossible to do many simultaneous comparisons, but dieldrin and γ BHC, which it was particularly interesting to compare, were investigated on flies from the same batch of pupae (A in the tables), using each insecticide on alternate days. DDT was used separately and a check on the susceptibility of this batch (B) to dieldrin was made by exposing 30 young males and 20 young females to $0.00084 \mu\text{g}$. dieldrin per fly. When the three common chlorinated-hydrocarbon insecticides had been fully evaluated, aldrin, endrin and Telodrin were tried. Only male flies were then available and, to economise in their use, fewer concentrations of the insecticides were applied than in the first tests. Because of batch variation (see below) a simultaneous check was made with dieldrin at $0.0017 \mu\text{g}$. per fly.

Results.

All mortalities are given after correction for control deaths (Fisher & Yates, 1948), which are also given. It was found that corrected mortalities for all insecticides tested, except γ BHC, increased up to 72 hours, after which observations were discontinued because of rapidly increasing control mortalities. With γ BHC, mortality was almost complete in 24 hours and complete in 48 hours, and this latter period is preferred to 72 hours for evaluation of γ BHC because control deaths are lower. It is, of course, legitimate to compare poisons only after such a period as will ensure that their effects are complete.

Effect of solvent.

It is possible that the presence of an involatile solvent might affect the lethality of the insecticide in a droplet picked up in the field. To obtain information on this, solutions of DDT, γ BHC and dieldrin in the volatile kerosene and involatile Risella 117 were tested in parallel. The results are given in Table I. DDT was tested at two rates of application and at each the mortality was the same for both

solvents. Dieldrin and γ BHC were each used at only a single concentration but they showed no difference in effect between the two solvents. It is most unlikely that this would occur if the slopes of the regression lines were different and it may be assumed that the presence of the involatile but non-toxic Risella oil did not affect the lethality of the insecticides. There was some evidence that the Risella 117 solutions were slower in action, and because this would be an experimental disadvantage owing to rapidly increasing control mortalities, this solvent was not used for the bulk of the experiments.

TABLE I.

Mortality amongst young adults of *G. morsitans* caused by insecticides applied in different solvents. Results expressed as mortality percentages, corrected for control deaths. Numbers of flies used shown in brackets.

	Insecticide and dosage (μg.)					
Solvent ..	Dieldrin	γBHC	Control	DDT		Control
	0.0017	0.0067	Nil	0.027	0.054	Nil
Kerosene ..	42 (33)	50 (26)	6 (33)	57 (30)	80 (30)	0 (10)
Risella 117 ..	39 (36)	46 (26)	12 (25)	57 (30)	81 (31)	0 (10)
	Male flies, tested June 1960			Female flies, tested Oct.–Nov. 1960		

Effect of insecticides.

Mortalities are listed in Tables II and III, and plotted on log-probit paper in figs. 1 and 2. Regression lines have been calculated and tested by the method of Litchfield & Wilcoxon (1949) and the results are listed in Table IV.

All lines are straight, there being no significant heterogeneity ($P=0.05$). For each insecticide, the lines for males and females do not differ significantly ($P=0.05$) as regards either slope or position. The three lines for dieldrin are statistically parallel to one another and to those for DDT and γ BHC, which, however, differ significantly one from the other. The three potency ratios differ significantly ($P=0.05$), although DDT and γ BHC cannot properly be compared because the slopes of their regression lines differ significantly. The effects of these two insecticides are so different, however, that a comparison must be made, if only in general terms. Because the slopes of the dieldrin lines do not differ significantly from those of any other lines, the potency of each insecticide has been compared with that determined for dieldrin in respect of batch C, the least susceptible batch (Table V).

Changes in susceptibility with time were encountered and for brevity this phenomenon is referred to as 'batch' variation, although this term is usually confined to insects exposed on a single occasion. Batch A included flies used for the initial determination of the regression lines for dieldrin and γ BHC. Batch B was used for DDT and its susceptibility to dieldrin was checked and found not to differ from that of batch A (Table II and fig. 1). Flies employed in later tests gave unexpected results, and a new regression line was determined for the males exposed to dieldrin (batch C), giving a susceptibility less than half that of batch A. This is too great a decrease to be explained by crude increase in body weight. When males of batch C were cross-checked against γ BHC, it was evident that susceptibility to this insecticide also was reduced; the mortality among 60 male flies was only 60 per cent. following a dose of 0.0067μ g., which is well outside the fiducial limits of the LD₆₀ for batch A (0.0029 – 0.0042μ g.). A second line for γ BHC was

TABLE II.

Mortality amongst young adults of *G. morsitans* after topical application of different insecticides in kerosene. Results expressed as mortality percentages after 72 hours (dieldrin and DDT) or 48 hours (γ BHC), corrected for appropriate control mortalities.

Insecticide	Sex	Batch	Dates	No. used	Mean wt. (mg.)	Dose per fly (μg.)								Control		
						0.00042	0.00084	0.0017	0.0034	0.0051	0.0067	0.0135	0.027		0.054	0.108
Dieldrin	♂	A	23.x-9.xi.59	155	24.1 ± 2.3	33	44	80	100	—	—	—	—	—	—	20
	♂	B	7.iv.60	30	—	—	65	—	—	—	—	—	—	—	—	15
	♂	C	22.vii-4.viii.60	126	29.2 ± 3.1	—	—	48	86	—	96.5	—	—	—	—	0
	♀	A	23.x-9.xi.59	203	29.1 ± 3.7	21	43	62	90	—	—	—	—	—	—	16
	♀	B	7.iv.60	20	—	—	43	—	—	—	—	—	—	—	—	8
γ BHC	♂	A	22.x-7.xi.59	159	24.1 ± 2.3	—	—	21	47	75	90	100	—	—	—	9
	♂	C	22.vii-4.viii.60	60	29.2 ± 3.1	—	—	—	—	—	60	—	—	—	—	0
	♂	D	24/29.xii.60	80	—	—	—	—	—	21	45	87	—	—	—	10
	♀	A	22.x-7.xi.59	151	29.2 ± 3.7	—	—	9	46	65	89	100	—	—	—	13
	♀	B	16.iii-9.iv.60	236	29.8 ± 4.0	—	—	—	6	—	24	45	60	88	100	15
DDT ..	♂	C	22.vii-5.viii.60	30	29.2 ± 3.1	—	—	—	—	—	—	—	70	—	—	0
	♂	B	16.iii-9.iv.60	223	34.2 ± 4.6	—	—	—	—	—	17	27	50	76	87	8

determined at the end of 1960 and was parallel to the earlier one, but the LD₅₀ increased 2.1 times, showing good agreement with the decrease in susceptibility to dieldrin. This is referred to as batch D. There was no change in susceptibility after the initial decrease and susceptibility to DDT remained unchanged throughout the investigation.

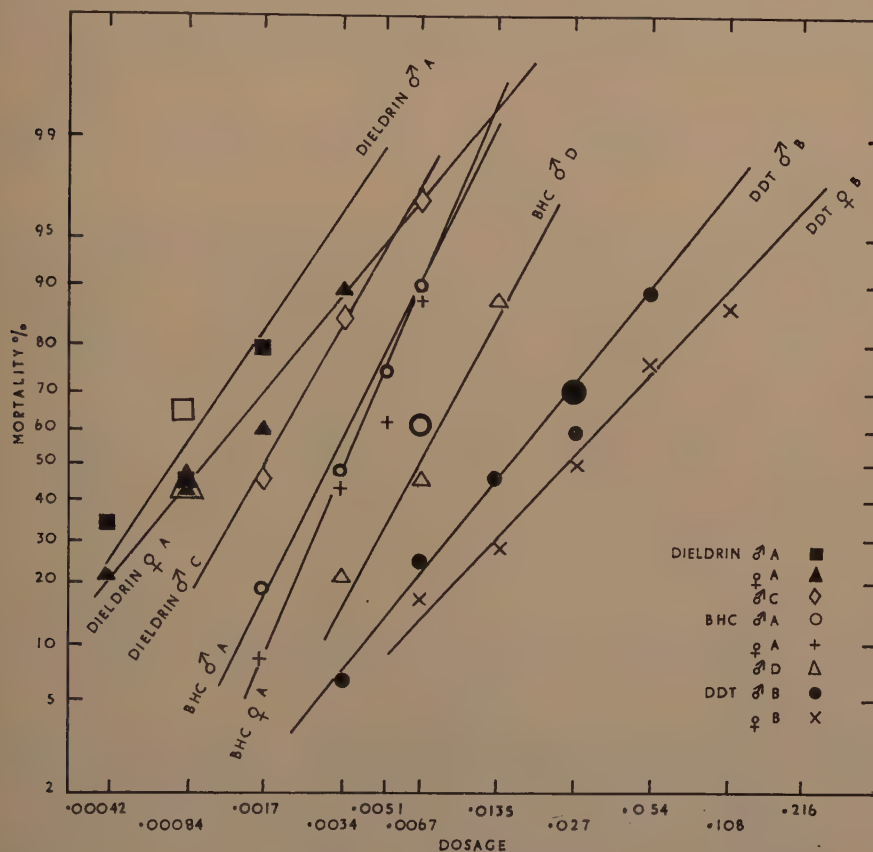


Fig. 1.—Log-dosage/probit-mortality regression lines for young adults of *G. morsitans* in respect of dieldrin, γ BHC and DDT (data from Table II). The large symbols represent the checks made with dieldrin on batch B (large square, ♂ ♂; large triangle, ♀ ♀), and with γ BHC (large open circle) and DDT (large solid circle) on males of batch C. Abscissae: dosages ($\mu\text{g.}$), plotted on logarithmic scale. Ordinates: mortality percentages, plotted on a linear scale of probits. Points for 100% mortality omitted, but their corrected values (*vide* Litchfield & Wilcoxon, 1949) were utilised in plotting the regression lines.

Checks were made as follows to determine whether the differences observed might be seasonal: to dieldrin in June 1960 (Table I), July–August 1960 (Table II, fig. 1), September 1960 (Table III, fig. 2), and October 1960 (when 12 out of 25 males died when treated with 0.0017 $\mu\text{g.}$ dieldrin); to γ BHC in July–August 1960 (Tables I, II; fig. 1) and December 1960 (Table II, fig. 1); and to DDT in July–August 1960 (Table II, fig. 1) and October–November 1960 (Table I, fig. 1). All tests were made on males, except the last, and owing to the virtual failure of the late rains of 1960, all were made in dry-season conditions.

TABLE III.

Mortality amongst young male adults of *G. morsitans* after topical application of aldrin, endrin, Telodrin and dieldrin. Results expressed as mortality percentages after 72 hours, corrected for control deaths. Mean wt. of flies, 26.1 ± 4.13 mg.

Insecticide	Date	No. used	Dose per fly (μ g.)						Control
			·00042	·00084	·0017	·00675	·0135	0·027	
Aldrin ..	31.viii—	66	—	—	—	20	48	80	11
Endrin ..	24.ix.60	30	—	—	42	—	—	—	11
Telodrin ..		90	29	64	96	—	—	—	11
Dieldrin ..		30	—	—	40	—	—	—	11

TABLE IV.

Dosage-mortality constants for young adults of *G. morsitans*.

Insecticide	Sex	Batch	LD50 (μ g.)	95% fiducial limits of LD50 (μ g.)	LD95 (μ g.)
Dieldrin	♂	A	0·00075	0·00057—0·00099	0·0029
		A	0·0010	0·00078—0·0013	0·0054
		C	0·0017	0·0013—0·0022	0·0051
γ BHC	♂	A	0·0030	0·0025—0·0036	0·0085
		D	0·0064	0·0047—0·0088	0·021
		A	0·0036	0·0030—0·0045	0·0087
p,p'DDT	♂	B	0·0165	0·0129—0·0212	0·073
		B	0·025	0·0184—0·0340	0·175
Aldrin	♂	C	0·0135	0·0103—0·0177	0·052
Telodrin	♂	C	0·00062	0·00050—0·00077	0·0017

TABLE V.

Potencies of insecticides in respect of young adults of *G. morsitans*, compared with potency of dieldrin determined for batch C, unless otherwise stated. Potencies compared at LD50 level.

Insecticide	Males	Females
Dieldrin (batch A)	2·3 (1·5—3·4)	—
γ BHC (batch A) compared with dieldrin (batch A)	0·25 (0·18—0·37)	0·28 (0·20—0·40)
γ BHC (batch D)	0·26 (0·17—0·40)	—
DDT	0·103 (0·071—0·15)	—
DDT, compared with dieldrin (batch A)	0·04 (0·027—0·056)	0·04 (0·027—0·06)
Aldrin	0·13 (0·085—0·19)	—
Endrin	circa 1	—
Telodrin	2·7 (1·9—3·9)	—

Figures in brackets show the 95 per cent. fiducial limits. All data are derived from Table IV, except value for endrin, which derives from Table III.

In all cases the mortalities are within the 95 per cent. fiducial limits for batch C (dieldrin), batch D (γ BHC) and batch B (DDT). There is no sign of any seasonal change. In fact the only changes noted were the falls in susceptibility to dieldrin and BHC between October–November 1959 (late dry season) and the next July–August (early next long dry season), but there was no return to the former level the next October and December. DDT showed no change between the rainy season of 1960 (March–April, Table II) and the following dry season (see above). It seems that the potencies of dieldrin and γ BHC are linked but independent of that of DDT.

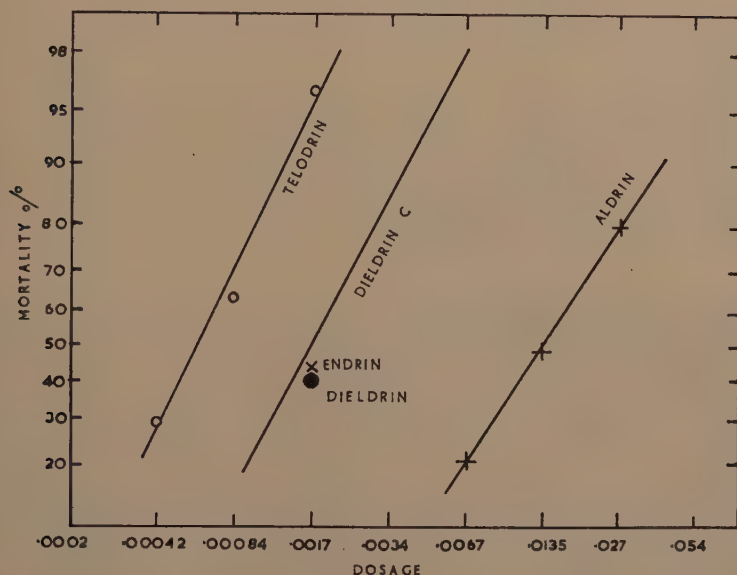


Fig. 2.—Log-dosage/probit-mortality regression lines for young male adults of *G. morsitans* in respect of aldrin and Telodrin (data from Table III). The line for dieldrin (batch C, males) is also plotted for comparison (data from Table II). The single test on endrin is represented by the cross and the simultaneous check on dieldrin by the solid circle (data from Table III). Abscissae: dosages ($\mu\text{g.}$), plotted on logarithmic scale. Ordinates: mortality percentages, plotted on a linear scale of probits.

Discussion.

G. morsitans appears to be unusually susceptible to chlorinated-hydrocarbon insecticides. Published figures of LD50 values for other Muscids exceed those given in Table IV; thus, with *Musca domestica* L., Hadaway (1956) found the values for DDT, γ BHC and dieldrin to be 0.45, 0.104 and 0.27 $\mu\text{g.}$, respectively. These tests were made with the same type of apparatus and solvent as those used in the present investigations. Rogoff & Metcalf (1951) found LD50 values for *M. domestica* of 0.033, 0.01, 0.031 and 0.035 $\mu\text{g.}$ for DDT, γ BHC, dieldrin and aldrin, respectively; Lewallen (1954) determined the LD50 values for *Fannia canicularis* (L.) for DDT, lindane and dieldrin to be 2.8, 0.76 and 0.003 $\mu\text{g.}$, respectively. Both species are smaller than the tsetse fly. The unusual susceptibility of *Glossina* here recorded is not due to the use of flies that had emerged in the laboratory (although tsetse are notoriously poor laboratory insects), for wild-caught flies of the very closely related species *G. swynnertoni* Aust. proved even

more susceptible than these *G. morsitans* (unpublished results). The relative potencies of the insecticides are not unusual. Busvine (1952) lists these for *M. domestica*, relative to DDT, as 3·3–17 for lindane, 7–11 for aldrin and 10–17 for dieldrin. Apart from those for aldrin, which is only about as effective as DDT against *G. morsitans*, these figures are comparable to those in Table V.

Present-day prices of dieldrin and γ BHC are about the same, and DDT is about one-third as expensive. It is apparent that dieldrin is the best insecticide to use on a cost basis and it has proved the best in practice for aerial spraying (Burnett & others, 1961). This may be partly because a lethal dose can be contained in a smaller drop than is necessary for other insecticides, which is better adapted to passing obstacles and then impacting on flies. Of the three toxicants that have not been used in the field, aldrin can be ruled out because of its low activity and endrin because it costs more than dieldrin, is no more active and is considerably more toxic to mammals. Telodrin is also thought to be of higher mammalian toxicity and its price, at present uncertain, may exceed that of dieldrin; with a probable application rate of only 6 g. per acre, mammalian toxicity may be unimportant, and it is the only insecticide that shows any promise of superseding dieldrin. The application of these findings to aerial operations against tsetse flies cannot be properly made until work in progress on pregnant females is complete; these seem to be considerably more resistant than the young flies.

Summary.

Solutions of six chlorinated-hydrocarbon insecticides in kerosene have been applied in drops of about 0·02 microlitre (μ l.) to adults of *Glossina morsitans* Westw., 2–5 days old, one day after the first meal. This species is found to be unusually susceptible to this group of insecticides. In order of increasing toxicity they are: DDT and aldrin, γ BHC, dieldrin and endrin, Telodrin (1,3,4,5,6,7,8,8-octachloro-3a,4,7,7a-tetrahydro-4,7-methanophthalan). The LD₅₀ of DDT was 0·0165 μ g. (males) and 0·025 μ g. (females), that of Telodrin was 0·00062 μ g. (males). Susceptibility of the two sexes to DDT, γ BHC and dieldrin did not differ significantly. Two batches of flies tested with an interval of eight months differed by about two times in their response to dieldrin and γ BHC, but the response to DDT was unchanged. This difference was not seasonal.

For practical use, dieldrin is the best and cheapest available insecticide, a fact confirmed in the field. Only Telodrin might replace it.

Acknowledgements.

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OVIPOSITION IN DDT-RESISTANT AND SUSCEPTIBLE STRAINS OF *Aedes aegypti* (L.) IN RELATION TO LIGHT PREFERENCE.

By R. J. Wood *

London School of Hygiene and Tropical Medicine.

A preliminary study on oviposition behaviour in *Aedes aegypti* (L.) (Wood, 1959) demonstrated a significant difference between DDT-resistant and susceptible strains. Resistant strains from Trinidad and Haiti, presented with the choice of ovipositing at sites illuminated at two different levels, laid more readily at the darker of these sites, that illuminated at 0.02 lumen per sq. ft., whereas with two susceptible strains, from West Africa and from U.S.A., the lighter site, at 6.5 lumens per sq. ft., was preferred. 48 132

The 'dark-laying' habit of the Trinidad DDT-resistant strain was particularly interesting in relation to a field investigation by Kellett & Omardeen (1957). They had found that, after some years of DDT larvicidal treatment in Trinidad, not only was this species strongly resistant to DDT (Gilkes, Kellett & Gillette, 1956), but it had also, so it seemed, increased its propensity for laying eggs in tree holes. There was clearly the possibility that all these three characters—'dark laying', tree-hole breeding and DDT resistance—might in some way be related to one another.

The present study has been designed as an attempt to answer three questions:—

Is 'oviposition light preference' genetically controlled and, if so, what is the mechanism involved?

Is there genetical association between 'dark laying' and DDT-resistance?

What significance has 'dark laying' in the Trinidad strain to the apparent increase in tree-hole breeding in Trinidad?

During the course of this work, knowledge has also increased on certain biological aspects of the problem.

Historical resumé.

Light and oviposition.

It has long been recognised that *A. aegypti* shows a preference for laying its eggs in shaded sites (Boyce, 1911; Buxton & Hopkins, 1927; Hopkins, 1952). Kennedy (1942) has shown that preference for shady places may be exercised by visits to more or less shady ones, but that the mosquito may also be attracted to places that appear dark (a scototaxis), the stimulus acting from a maximum distance of 20 cm. Both mechanisms are said to be equally important but the latter is supposed the more efficient. The surface reflectance of the site is also considered a critical factor (Kennedy, 1942; Beckel, 1955; O'Gower, 1957), a dark surface being more attractive.

In the classic experiments of Buxton & Hopkins (1927) in Samoa, *A. aegypti* chose the darkest of three experimental sites, the most favoured light intensity being one-twentieth of bright sunlight (10–20 lumens per sq. ft. ?); light intensities lower than this were not investigated. In addition, there have been three investigations, by Dunn (1928) in Nigeria, Smith (1956) on an island in

* Now at Istituto di Zoologia, Università, Pavia, Italy.

Lake Victoria and Surtees (1960), again in Nigeria, in which light has probably been a critical factor but from which other factors such as size, temperature, degree of shelter and the presence of organic matter cannot be excluded. Dunn (1928) compared the infestation in the field of two tree holes, one "basin-shaped" and the other "bottle-shaped" and found that the former, more open cavity was preferred. Smith (1956) noticed many larvae of *A. aegypti* in a tree hole illuminated at 2 lumens per sq. ft. while in a nearby rock pool at 60 lumens per sq. ft. there were no *A. aegypti*. Surtees (1960) found 50 per cent. of 200 shaded sites with larvae of *A. aegypti* as compared with only 20 per cent. of 63 he had placed in the open. All would seem to suggest that the shaded (but perhaps not the very dark) site is preferred.

While the moderately shaded site is perhaps the 'ideal' situation, natural conditions may be such that sites of an optimum nature are not always available. Then it seems that *A. aegypti*, notably a very adaptable mosquito, may lay eggs at sites illuminated at almost any level. Jobling (1937) has shown that under laboratory conditions *A. aegypti* is able to mate and oviposit in almost complete darkness, which explains how it is also able to breed, at times, in dark cellars and the dark interiors of cisterns. Chandler (1956) has noted that treatment of sites of this type, easily overlooked by the perifocal sprayer, was especially important for the control of *A. aegypti* in urban areas of Texas.

Some breeding at brightly-lit sites under field conditions has been noted by Surtees (1960). Under laboratory conditions it is well known that *A. aegypti* will oviposit at sites illuminated to quite high levels but no measurements seem to have been recorded. The author has noted apparently normal oviposition at sites illuminated at 30 lumens per sq. ft.*; but almost certainly much higher light intensities are also tolerated.

It may be noted that, in complete darkness, *A. aegypti* is at some disadvantage. Not only is egg-laying delayed (Haddow & Gillett, 1957) and the adult generally less active (Kennedy, 1940; Haddow & Gillett, 1957) but there is also evidence from an investigation by Galliard (1958) that hatching is delayed (in both a long-established and a new colony), that development from egg to adult is prolonged (also in both colonies) and that the level of mortality during development is much higher than normal (only in the new colony).

Sites of this extreme type will possibly assume importance only under conditions of stringent control, with the treatment or destruction of more conventional breeding places. Whether oviposition at very brightly-lit sites also holds a selective disadvantage is not recorded.

It is unfortunate that the level of illumination at breeding places of *A. aegypti* in Trinidad was not measured. However, Omardeen (personal communication, 23rd March 1959) mentions that most tree holes examined were of the "basin-type", rather than the "bottle-type" of Dunn (1928), i.e., wider at the top and narrowing down to the depths, although in many cases they were of rather a different shape from the example given by Dunn (1928), their depth being several times as great as the diameter of their opening. Indeed they were mostly so dark that a flashlight proved necessary to see inside them, which would seem to suggest a much lower level of illumination than that indicated from the measurements of the "basin-hole" described by Dunn (1928).

So far in this account the effect of light on oviposition has been pictured as a direct stimulus, albeit a complex one, on the gravid female causing her to choose between sites illuminated at different levels of intensity. That this is not the only effect which light exerts is apparent from the studies of Haddow & Gillett (1957), Gillett, Haddow & Corbet (1959) and Gillett, Corbet & Haddow (1959) who have shown the stimulus of light to be equally important as the

* This and other light measurements were made with an Everett-Edgumbe Auto-Photometer.

instigator of a daily oviposition rhythm. This rhythm can be broken only by exposure of the female adults to constant light or darkness and is not affected by the conditions of light intensity under which larvae and pupae have been reared.

Previous experiments.

In the earlier investigation (Wood, 1959), each of four strains (two DDT-resistant and two susceptible to DDT) was compared in a standard test cage, 18 in. square, divided by a partition into two interconnecting compartments, one of which was blacked out, and the other exposed to constant illumination from an artificial source. Mosquitoes had the opportunity to lay eggs at an oviposition site in either compartment, in both of which conditions of temperature* and humidity† were constant and identical. The two sites were illuminated, respectively, at 0.02 and 6.5 lumens per sq. ft. Adults to be tested emerged in the test cages and from the moment of emergence were subject to conditions of constant light. (Here it was assumed that even on entering the dark compartment they were still stimulated by the presence of the bright connecting slit through which they had passed.) Thus it was considered very improbable that an oviposition cycle had been set up in the manner reported by Haddow & Gillett (1957) and the results were interpreted as a simple choice between the two sites.

Experimental material.

In the present investigation seven strains were used, three DDT-resistant and four susceptible.

The Normal susceptible strain (NS) came from the stock colony of the Department of Entomology, London School of Hygiene and Tropical Medicine and was started with eggs collected in West Africa 30 years ago.

The American susceptible strain (AS), supplied by Dr. G. B. Craig of the University of Notre Dame, Indiana, was originally obtained from the National Institutes of Health, Bethesda, Maryland, where it had been under laboratory culture for at least 25 years.

The 'Queenslandensis' susceptible strain (QS) had a history of some seven years in the Department of Parasitology, London School of Hygiene and Tropical Medicine since it was started with eggs sent from Jeddah, Saudi-Arabia, by Dr. D. J. Lewis. This strain was probably homozygous for colour characters typifying *A. aegypti* var. *queenslandensis* (Theo.).

The Karankasso susceptible strain (KS) kindly supplied by Dr. J. Hamon in June 1958 had been started two months earlier from material collected at Karankasso, a village 60 miles from Bobo Dioulasso, Haute Volta, French West Africa, and never subject to the action of insecticide.

The Trinidad resistant strain (TR) was started with eggs sent from the Communicable Disease Centre, Savannah, Georgia in 1956. It had then been under laboratory culture for about two years. Larvae of this strain were about 120 times resistant to DDT (Coker, 1958).

The Selected Trinidad resistant strain (STR), already DDT-resistant when collected in the field in 1954 (=TR) but later further selected with DDT at the 95 per cent. level for 13 generations, was supplied by Dr. G. B. Craig. Larval resistance to DDT exceeded 2,500 times (Craig, unpublished).

The Haiti resistant strain (HR), started with eggs sent by Dr. Aldighieri in 1954, has been investigated by Coker (1958) and shown to have a larval resistance to DDT of 350 times.

* Measured with a standard mercury-and-glass thermometer.

† Measured with an Edney paper hygrometer, recalibrated with a standard-type whirling hygrometer.

More detailed evidence with regard to resistance levels is summarised in Table I. The authorities quoted have used techniques similar to those now adopted by the WHO; *i.e.*, larvae are exposed for 24 hours to the insecticide in aqueous suspension, and adults are confined next to DDT-impregnated paper for 1 hour, with a 24-hour holding period.

TABLE I.
Resistance levels of experimental strains.

Strain	LD50 for DDT		Authority
	Larvae (in p.p.m.)	Adults (in per cent.)	
NS	0.03	0.9	Shidrawi (1957)
	0.016	—	Coker (1958)
	0.03	—	Qutubuddin (1958)
AS	0.008	1.4	Craig (unpublished)
QS	0.02	1.4	Shidrawi (1957)
KS	—	0.9—1.0	Wood (unpublished)
TR	4.0	—	Fay (1956)
	2.0	—	Brown & Perry (1956)
	2.0	4.2	Coker (1958)
	0.5	—	Craig, Wood (unpublished)
STR	20.0	—	Craig (unpublished)
	30.0	—	Qutubuddin (1958)
HR	5.8	—	Coker (1958)

TABLE II.

The relative attractiveness of two oviposition sites, illuminated at different levels, to gravid females of DDT-resistant and susceptible strains of *Aedes aegypti*.

Strain	Experiment	Cage	No. of eggs laid in light	No. of eggs laid in dark	Total	Percentage of eggs laid in dark	Mean \pm standard error
Selected Trinidad resistant	1	B	570	1963	2533	77.6	65.2 \pm 2.8
	2	B	1537	2188	3725	58.4	
	3	B	1018	1174	2192	52.6	
	4	B	622	921	1543	59.7	
	5	B	502	1007	1509	66.7	
	6	B	785	1713	2498	68.9	
	7	B	652	1520	2172	69.9	
	8	A	657	1396	2053	67.9	
Haiti resistant	1	A	1418	1760	3178	55.4	50.2 \pm 5.7
	2	A	1205	986	2191	44.9	
American susceptible	1	A	2092	325	2417	13.4	17.4 \pm 3.3
	2	B	751	136	887	15.5	
	3	A	1072	582	1654	35.2	
	4	A	992	102	1094	9.3	
	5	B	1247	147	1394	10.5	
	6	A	1761	446	2207	20.2	
Normal susceptible	1	A	1319	694	2013	33.8	30.3 \pm 5.7
	2	B	3482	1278	4760	26.8	
Karankasso susceptible	1	B	522	1007	1529	65.8	53.5 \pm 5.7
	2	A	339	239	578	41.3	

Comparison of strains.

The same apparatus as that referred to above (cages 'A' and 'B') and more fully described by Wood (1959) was used for an investigation on the wild-caught African strain Karankasso susceptible (KS) and for further studies on the Selected Trinidad resistant (STR) strain.

In Table II are summarised all the results so far obtained using this apparatus. In the four strains with some years' laboratory history (STR, HR, AS, NS) clear differences in 'oviposition light preference' can be appreciated between those DDT-resistant and those DDT-susceptible. These differences are statistically significant. Results from the Karankasso susceptible strain, however, seem to deny any connection between 'dark laying' and resistance, the results from this strain differing in no significant way from those of the two resistant colonies. The KS strain, it may be noted, included a rather large proportion of forms which could only be described as var. *queenslandensis* (see Mattingly, 1957), and in this respect differed from the NS and AS strains which seemed to be composed only of the type form, but resembled the STR colony. A more detailed morphological comparison of the STR and KS strains suggested a greater proportion of 'pale' forms (var. *queenslandensis*) in the latter, the incidence of 'pale' forms in the STR strain being slight.

In a further series of tests, five strains: 'Queenslandensis' susceptible, American susceptible, Karankasso susceptible, Trinidad resistant (unselected) and Selected Trinidad resistant were compared in a third cage (cage C), the light half of which was illuminated at 3.5 lumens per sq. ft. and the dark half at an intensity well below the sensitivity of the measuring instrument (less than 0.02 lumen per sq. ft.).

TABLE III.

Oviposition by five strains in cage C.

Strain	Experiment	No. of eggs laid in light	No. of eggs laid in dark	Total	Percentage of eggs laid in dark	Mean \pm standard error
Trinidad resistant	1	539	344	883	39.0	41.8 \pm 2.9
	2	1651	1090	2741	39.7	
	3	1893	1216	3109	39.1	
	4	1153	1133	2286	49.6	
Selected Trinidad resistant	1	1199	862	2061	41.8	51.6 \pm 3.3
	2	622	921	1543	59.7	
	3	366	420	786	53.4	
American susceptible	1	2888	173	3061	5.6	4.05 \pm 4.05
	2	1350	35	1385	2.5	
Queenslandensis susceptible	1	1167	512	1679	30.5	33.2 \pm 4.05
	2	455	255	710	35.9	
Karankasso susceptible	1	541	231	772	29.9	29.9 \pm 5.7

Results which are given in Table III show that in all strains a lower proportion of eggs was laid in the dark. The mean values for the STR and TR strains, which are not significantly different from one another, are clearly different from those of the AS strain. The QS and KS strains, with similar values, are intermediate. It may be noted that the 'Queenslandensis' strain probably consisted entirely

TABLE IV.
The percentage of males and females resting in each half of a divided test cage (A or B).

Strain	No. replicates	No. of adults resting in light half		No. of adults resting in dark half		Percentage resting in dark		Variation (%)	
		♀	♂	♀	♂	♀	♂	♀	♂
STR	14	307	345	53	19	14.7	5.2	0-45.0	0-20.0
AS	5	70	31	12	5	14.6	13.9	10.0-22.2	0-26.7
F ₁ (AS × STR)	3	57	79	12	9	17.4	10.2	11.1-26.9	0-18.9

of var. *queenslandensis* but unlike the Karankasso strain had had several years of laboratory history.

Before discussing the significance of these results and those in Table II, it is worth examining oviposition in more detail. Particular attention will be drawn to the selection of the oviposition site by the gravid female.

Preoviposition behaviour.

To simplify matters, only the two extreme cases, the AS and STR strains, will be considered. Furthermore it will be assumed that light alone was the controlling factor: all other influences known to affect oviposition, *e.g.*, wind, humidity, and intrinsic factors at the site, were either excluded or were constant and equal on both sides of the test cage.

Investigation of resting habits in the test cages A and B revealed a very similar pattern of behaviour in the two strains. Males and females of both strains tended to rest principally in the upper half of the light side of the test cage (illuminated at 10–11 lumens per sq. ft.), and to a lesser extent nearer the floor on the light side (11–12 lumens per sq. ft.), and in the dark side close to the connecting slit (0.05–0.010 lumen per sq. ft.). Very few rested near the floor of the cage on the dark side (0.02 lumen per sq. ft.). Counts of those resting on each side of the cage are given in Table IV.

Resting was assessed between 11 a.m. and 5 p.m., approximately one week after the first blood-meal, *i.e.*, when the females had laid one egg-batch and were ready to feed again. Generally a somewhat greater percentage of females (mean 15.1%) rested in the dark than that of males (mean 6.8%). A similar situation was also found in cage C.

For a mosquito to lay in the dark half of a divided test cage, it must usually have made a special journey there from its resting place in the light half. Kennedy (1942) has shown that the first part of preoviposition behaviour in *A. aegypti* is a tendency to fly nearer the floor; but he maintains that this may be upset by the intervention of other stimuli. It is easy to see that the dark connecting slit, less than 20 cm. from the majority of resting females and therefore able to evoke a directional response from them (Kennedy, 1942), could have been just such a scototactic stimulus to the STR strain. It is supposed that most females of the AS strain, on the other hand, must either have been indifferent to, or actively repelled by, this stimulus.

The alternative is that the most favoured site—or the situation of this site—was itself attractive and that exploratory activity between the two sites took place preliminary to one or another being chosen for oviposition. Evidence which follows suggests that exploratory activity of this kind probably occurred in the STR strain for at least some hours (>24) after the first blood-meal. Further evidence indicates that attraction to the site itself was probably not the critical factor, but rather the dark place in which the site was situated.

Oviposition period.

Counts of eggs laid on the two sides of test cages during the first and subsequent days following the first blood-meal indicated that the 'dark-laying' STR strain tended to lay a lower proportion of its eggs (42.5%) at the darker site on the initial day of oviposition than it did on subsequent days (Table V, fig. 1). By the second day, the percentage laid in the dark had risen to 69 per cent., and this situation was maintained during the next three or four days, after which the proportion on the dark side declined again. In the AS strain, which laid most of its eggs over three days, the relative proportion on the two sides of the cage did not change.

TABLE V.
The daily proportion of eggs laid in the dark half of test cages A and B by the STR and AS strains following a single blood-meal.

Strain	Dark or light side	Numbers of eggs laid on day*									No. of replicates	Total
		2	3	4	5	6	7	8	9	10		
AS	D	1097	1193	389	0	—	—	—	—	—	8	2679
AS	L	4314	5413	1731	234	—	—	—	—	—	8	11692
Totals		5411	6606	2120	234	—	—	—	—	—	16	14371
Percentage in the dark		20.27	18.06	18.35	0.00	—	—	—	—	—	—	—
STR	D	1276	7839	4321	2862	857	492	328	255	—	14	18230
STR	L	1727	3534	2191	1244	454	434	286	0	—	14	9870
Totals		3003	11373	6512	4106	1311	926	634	255	—	28	28100
Percentage in the dark		42.49	68.93	66.35	69.70	65.37	53.13	48.26	100	—	—	—

* First blood-meal given on day 0.

It will be apparent that these results also indicate another difference between these two strains, *i.e.*, in the length of their oviposition cycle. The relation of this difference to the one under discussion will form the subject of a later paper.

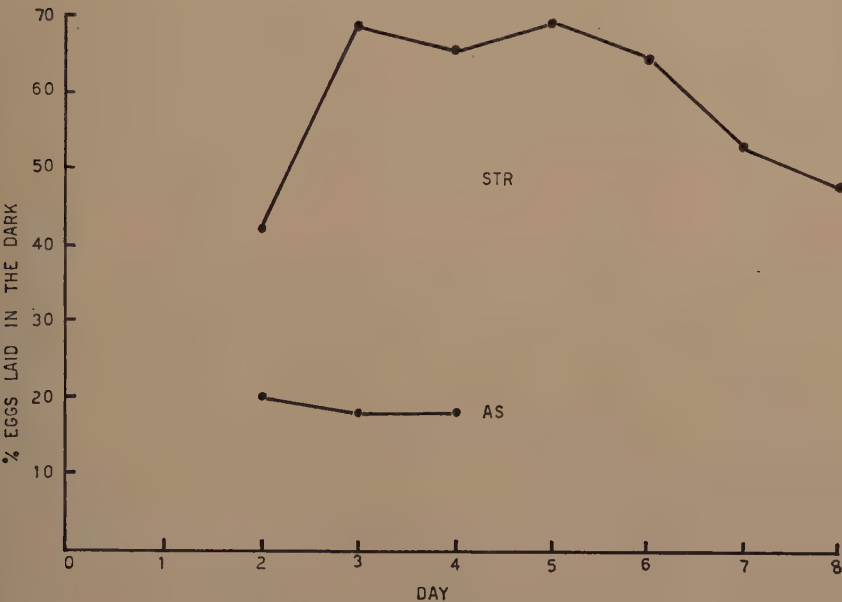


Fig. 1.—Daily percentage of eggs laid in the 'dark half' of the test cage following blood-meal on day 0.

The reflectance of the site.

To decide whether the attractiveness of the particular site was due to its own reflectance, the two strains of AS and STR, confined in undivided stock cages, were given the choice of ovipositing at sites of two contrasting types. Both sites were composed of a cone of filter paper inverted into a 100-cc. beaker filled three-quarters full with tap-water, but while in one case the filter paper was white, in the other it was black. Each strain was represented by ten females and five males and several blood-meals were given. Results are shown in Table VI; in both strains the black site was much preferred, but in the STR strain (with 85 per cent. at the black site) this preference was somewhat less well marked than in the AS strain (with 99%). Clearly it is not the reflectance from the site which is of prime importance; indeed here is strong evidence for the attraction to 'darkness,' *per se*, whether by a direct scototactic response or as the result of exploratory flights.

TABLE VI.
Oviposition at sites of contrasting reflectance.

Strain	No. of eggs laid		Percentage eggs laid on black site
	Black site	White site	
AS	673	8	98.83
STR	907	164	84.69

TABLE VII.

The oviposition preferences of four strains after their first and subsequent blood-meals.

Strain	Percentage eggs laid in the dark after blood-meal					
	1	2	3	4	5	6
STR ..	77.6	74.8	60.9	—	—	—
	58.4	83.7	77.3	70.2	—	—
Mean ..	68.0	79.25	69.1	70.2	—	—
HR ..	55.4	56.1	52.8	—	50.2	43.8
	44.9	50.6	51.1	—	—	—
Mean ..	50.15	53.35	51.9	—	50.2	43.8
AS ..	13.4	42.5	53.3	55.5	—	—
	15.5	59.8	47.6	—	—	—
Mean ..	14.45	51.15	50.45	55.5	—	—
NS ..	33.8	67.2	66.1	—	—	—
	26.8	44.4	34.6	—	37.1	22.5
Mean ..	30.3	55.8	50.35	—	37.1	22.5

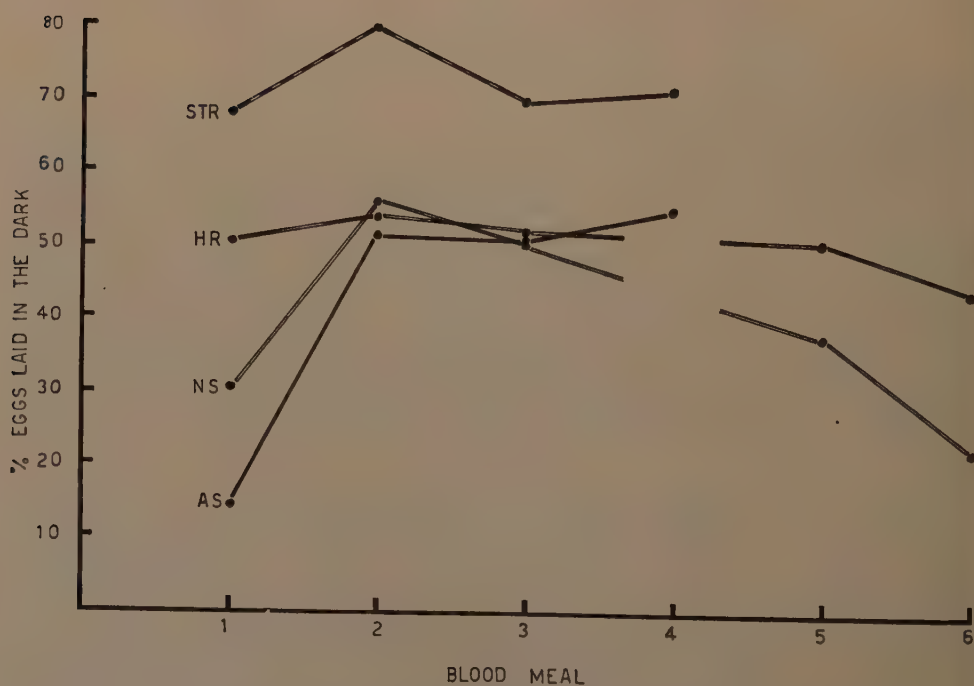


Fig. 2.—Oviposition by four strains after the first and subsequent blood-meals (combined results).

The effect of age.

As a further step towards understanding this strain difference, it is of value to ascertain whether the same oviposition preference is maintained throughout the life of a given batch of females. The results in Table VII and fig. 2 indicate that this is not necessarily so. Strains were examined for their oviposition preference in cages A or B after the first and subsequent blood-meals, with seven days elapsing between feeds. All four strains tended to deposit more eggs in the 'dark side' after their second blood-meal; indeed, after this and subsequent blood-meals, the AS, NS and HR strains laid approximately equal numbers in the dark as in the light (although with the limited number of replicates this cannot be certainly established for the NS strain). In contrast to this, the STR strain consistently laid a greater proportion of its eggs in the dark.

Genetics.

The results recorded in Tables II and III were collected over a period of 18 months during which time individual strains showed a marked uniformity of behaviour. To investigate the genetical mechanism involved, a number of mass reciprocal crosses and some back-crosses, using 10–20 individuals of both sexes, were made between the AS and STR strains. The results of testing them in the divided test cages A and B are given in the Table VIII. A comparison of these results (means) with those in Table II, demonstrates that the AS condition, 'light laying' shows dominance over 'dark laying,' characteristic of the STR strain. In the final two columns of Table VIII, the experimental results are compared with those which might be expected on the assumption that 'light laying' is controlled by a single dominant factor (see Appendix). It will be seen that resemblances are close, especially in the F_1 and F_2 , although there is considerable variation between replicates. The back-cross results are less concordant, but here again the mean value for the back-cross to STR male is close enough to the expected value to be significant.

In spite of a high variation between replicates, the mean values are in reasonable agreement with what would be expected on the basis of single-factor inheritance. It may be tentatively concluded that the mechanism is genetically simple.

It is of interest that McEwen (1918), reporting a case of insensitivity to light in *Drosophila*, describes one strain characterised by the mutant 'tan' which was "indifferent to light," i.e., failed to show the degree of phototropism exhibited by normal flies, although fully as active and with no apparent histological abnormality in the eyes. However, in this case the single factor involved was sex-linked to the female. Phototropism in *Drosophila* has also been attributed to a multi-factorial mechanism (Barigozzi & Tonissi, 1946).

Laying habit and DDT-resistance.

To investigate the possibility of a genetic connection between 'dark laying' and DDT-resistance, experiments took three forms:— (a) an attempt at selection for 'dark-laying' and 'light-laying' individuals within a single strain (STR); (b) comparison of the laying habits of the Trinidad resistant and the (DDT-) Selected Trinidad resistant strains; (c) further genetical studies with the Selected Trinidad resistant and American susceptible strains.

Selection for 'dark laying' and 'light laying.'—Preliminary experiments suggested that it might be possible to select, within a particular strain, for 'dark-laying' and 'light-laying' individuals: a single blood-fed female, when confined in one of the test cages (A or B, or a similar cage*), usually laid its complete egg-batch on one or other side of the cage, and did not usually divide it between the two.

* These cages, although smaller than A and B, reproduced the same conditions.

TABLE VIII.

The relative attractiveness of two sides of a 'divided test cage' to females of reciprocal crosses and back-crosses between the AS and STR strains.

Cross	Experiment	Cage	No. of eggs in light	No. of eggs in dark	Total	Percentage eggs in dark	Mean \pm standard error	Expected value
F_1 (AS \varnothing \times STR δ) ..	1	B	2316	297	2613	11.4	17.7 \pm 7.2	17.4 \pm
	2	B	2194	500	2694	18.5		
	3	A	1336	403	1739	23.2		
F_1 (STR \varnothing \times AS δ) ..	1	A	1857	887	2744	32.3	22.1 \pm 7.2	17.4 \pm
	2	A	2370	430	2800	15.3		
	3	B	553	126	679	18.6		
F_2 (AS \varnothing \times STR δ)	1	A	1338	719	2057	34.9	28.0 \pm 7.2	29.3 \pm
	2	B	1048	100	1148	8.7		
	3	B	400	273	673	40.5		
F_2 (STR \varnothing \times AS δ)	1	B	1988	395	2383	16.5	31.8 \pm 7.2	29.3 \pm
	2	A	2226	1077	3303	32.6		
	3	B	1127	970	2097	46.3		
F_1 (AS \varnothing \times STR δ) \varnothing \times STR δ	1	B	1596	1834	3430	53.4	45.8 \pm 5.6	41.3 \pm
	2	A	1279	1662	2941	56.5		
	3	A	1931	1144	3075	37.2		
	4	B	536	578	1114	51.9		
	5	A	708	307	1015	30.2		
F_1 (AS \varnothing \times STR δ) δ \times STR \varnothing	1	A	356	1162	1518	76.5	76.5 \pm 12.4	41.3 \pm
F_2 (F_1 (AS \varnothing \times STR δ) δ δ \times STR \varnothing)	1	B	708	521	1229	42.4	52.4 \pm 8.8	29.3 \pm
	2	A	396	659	1055	62.4		

Only on one occasion out of 13 were eggs found at both oviposition sites. Each egg-batch was laid over a period of 1-3 days.

The procedure for selecting 'dark-laying' and 'light-laying' females was as follows. Twenty blood-fed females of the STR strain were allowed to lay in one of the test cages, and their eggs, collected in the dark and the light, were used to start two new lines: in the 'dark-laying' line, eggs laid in the light were discarded in each generation; whereas in the 'light-laying' line, eggs laid in the dark were discarded. Selection proceeded over seven generations. Results are shown in Table IX and fig. 3.

TABLE IX.

The effect of selecting for 'dark laying' and 'light laying' in the Selected Trinidad resistant strain.

Strain	No. of eggs in the dark	No. of eggs in the light	Total	Percentage in the dark
STR	1174	1018	2192	52.6
STR-L	1396	657	2053	67.9
STR-D	1520	652	2172	69.9
STR-L ²	2152	848	3000	71.7
STR-D ²	high mortality during test			
STR-L ³	1146	724	1870	61.1
STR-D ³	1181	771	1952	60.5
STR-L ⁴	723	175	898	80.5
STR-D ⁴	1252	890	2142	58.4
STR-L ⁵	840	299	1139	26.3
STR-D ⁵	1873	2287	4160	45.02
STR-L ⁶	1202	577	1719	67.5
STR-D ⁶	829	870	1699	48.8
STR-L ⁷	419	1253	1672	22.4
STR-D ⁷	724	960	1684	43.0

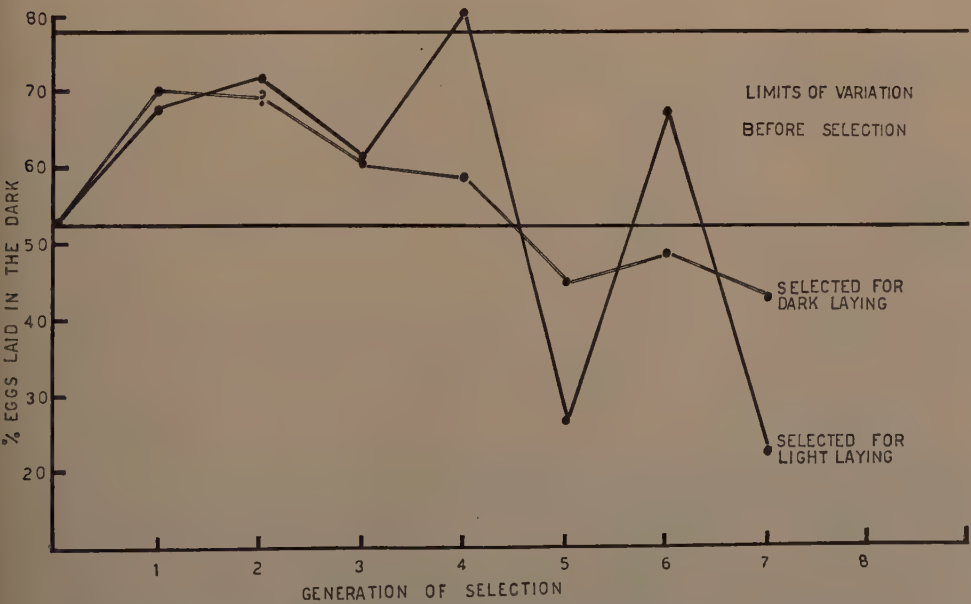


Fig. 3.—The effect of selecting for 'dark laying' and 'light laying' in the Selected Trinidad resistant strain.

In seven generations there has been no selection in the two directions expected. In fact both strains have tended towards ovipositing more in the light as selection has proceeded.

This inability to select for both 'dark-laying' and 'light-laying' substrains may well reflect the essential homogeneity of the STR strain (as is also suggested from the results of the crosses with the AS strain discussed in the previous section). It seems improbable that we have here a collection of individuals, some 'light layers' and the others 'dark layers.' It is more likely that this strain is genetically homogeneous for a tendency to lay at sites illuminated at certain levels. Thus, although individuals may sometimes vary in oviposition habit from one blood-meal to the next (there is some direct evidence for this), the general tendency of the strain remains constant. That both lines have reverted to the 'light-laying' condition may have been the result of successive generations of mild inbreeding. This is discussed more fully below.

The resistance of the STR strain to DDT was measured before, during and after selection, *i.e.*, over a period of 12 months. Using the WHO adult test kit, blood-fed females were exposed to a dose of 8 per cent. DDT for two hours, and then held for a further 24 hours. Results, which are given in Table X, show that after eight generations of selection for 'dark laying' and 'light laying' no difference in the level of DDT-resistance could be detected in the two lines. It may also be noted as a matter of interest that there had been no falling off of resistance during this 12-month period. Indeed, there would seem to have been a slight increase. Parallel instances of an increase in DDT tolerance in the absence of the insecticide have been recorded in several other species of mosquitos, including *Anopheles quadrimaculatus* Say (Ludvik, 1952; Hawkins, 1956), *A. labranchiae atroparvus* van Thiel (Mosna, 1957) and *Culex pipiens fatigans* Wied. (Newman, Aziz & Koshi, 1949).

TABLE X.

Exposure of fed females of the Selected Trinidad strain before, during and after 'oviposition selection', to deposits of 8 per cent. DDT for 2 hours.

Test material	No. of replicates	No. of females alive	No. of females dead	Total	Percentage mortality
STR (before 'oviposition selection')	6	61	53	114	46.5
STR-L ¹⁻⁶	7	47	46	93	49.4
STR-D ¹⁻⁶	6	45	17	62	27.4
STR-L ⁸	2	24	2	26	7.69
STR-D ⁸	2	30	6	36	16.67

The laying habits of the Trinidad resistant (unselected) and the (DDT-) Selected Trinidad resistant strains.—In a second line of investigation a comparison was made between the oviposition behaviour of the STR and TR strains. Clearly, should there be any genetic connection between oviposition behaviour and resistance, the strongly DDT-selected STR strain might well show a greater propensity for 'dark laying'. Reference to Table III will indicate that there was little difference between the two strains. Such difference as existed was not significant at the 5 per cent. level.

Genetical studies with Selected Trinidad resistant and American susceptible strains.—Reciprocal crosses were made between the STR and AS strains. The F_2 resulting from the cross $AS\varphi \times STR\sigma$ was investigated in one of the test cages (Table XII) and F_3 larvae reared from eggs laid in each half of the cage were examined for their resistance to DDT.

Tests were performed with the WHO larval test kit. The larvae were subjected to a dose of 10 p.p.m. for 24 hours, after which mortality was assessed. Qutubuddin (1958) has shown that this dose should kill 75 per cent. of the F_2 ($NS\varphi \times STR\sigma$), i.e., all heterozygotes and homozygous susceptibles. The results are given in Table XI. It can be seen that the resistance of the two lots of larvae

TABLE XI.

Tests with DDT on larvae derived from eggs laid by the F_2 ($AS\varphi \times STR\sigma$) in the two sides of a 'divided test cage'.

Material	No. of replicates	No. of larvae alive	No. of larvae dead	Percentage mortality
F_2 ($AS\varphi \times STR\sigma$) 'light side' ..	4	44	33	42.86
F_2 ($AS\varphi \times STR\sigma$) 'dark side' ..	2	21	17	44.74

did not differ. The survivors from these tests (presumably containing a high percentage of homozygous resistants) were then examined in a test cage for their oviposition behaviour. It will be clear from Table XII that there was no increase in 'dark laying,' indeed quite the reverse.

TABLE XII.

The relative attractiveness of the two sides of a 'divided test cage' to the F_2 ($AS\varphi \times STR\sigma$), and to the F_3 ($AS\varphi \times STR\sigma$) previously selected with 10 p.p.m. DDT at the larval stage.

Material	No. of eggs laid in light	No. of eggs laid in dark	Percentage eggs laid in dark
F_2 ($AS\varphi \times STR\sigma$) ..	1338	719	34.9
F_3 ($AS\varphi \times STR\sigma$) ..	748	193	20.5

It must be concluded from all these results that there is no obvious genetical connection between DDT-resistance in *A. aegypti* and a tendency to lay in very dark situations.

Discussion.

The Selected Trinidad DDT-resistant strain which showed the greatest tendency of seven strains towards laying eggs at dimly-lit sites, laid most readily at sites illuminated between <0.02 and 3.5 lumens per sq. ft., although sites illuminated at 6.5 lumens per sq. ft. were always utilised by a minority of individuals. In contrast to this, the AS strain laid most readily at the brighter sites and, to this strain, sites illuminated at 0.02 lumen per sq. ft. seemed generally unattractive (at least after the first blood-meal). The behaviour of other strains came within these two extremes.

It is of interest that even the STR strain seemed to lose the power to discriminate between sites at comparatively high levels of light intensity. Sites illuminated at 3.5 lumens per sq. ft. and <0.02 lumen per sq. ft. were equally attractive (Table III). This may be contrasted with the findings of Muirhead Thomson (1940) working with *Anopheles minimus* Theo. This mosquito always preferred the darker of two oviposition sites even when the light intensity was extremely low. The power to discriminate was lost only when the light intensity was down to 0.000007 lumen per sq. ft.

For resting, light intensities above 0.10 lumen per sq. ft. and below 12 lumens per sq. ft. were preferred by all strains. This is in agreement with the field investigation of Smith (1956) who found more adults of *A. aegypti* resting in bushes with dark interiors (at 2 lumens per sq. ft.) than in the others illuminated at 20 lumens per sq. ft.

The absence of inter-strain differences in resting behaviour is interesting. Assuming that inter-strain variations reflect an over-all difference in phototropism, it must be concluded that resting behaviour is not so critically controlled (*i.e.*, is not confined to such narrow limits) by light intensity as is oviposition.

An interesting point has arisen in discussing this work with Mr. P. F. Mattingly. Commenting on my inability to select for 'dark-laying' and 'light-laying' sub-strains, he suggests that these strains might have differed not in a simple tendency to lay in the 'light' or the 'dark' but in a compound behaviour pattern resulting from oviposition rhythm coupled perhaps with a cycle (differing between strains) of regular migration in and out of the dark side of the test cage. In the absence of precise tests for the presence of oviposition and activity cycles it is not possible to do more than comment on this theory. Nevertheless it would seem worth considering what evidence there is. Evidence with respect to resting behaviour indicates that between 11 a.m. and 5 p.m. there was no significant variation in the proportion of mosquitos (of any strain) resting on the two sides of the cage; whether a change occurred at 'night' is not known but it seems unlikely that an 'in-out' cycle would necessarily correspond with the change from day to night in the 'outside world' of which the adult mosquitos would have no experience. The suggestion that an oviposition rhythm might be operating is more easily countered. All strains were from the moment of emergence subject to continuous exposure to light and this, from the evidence of Haddow & Gillett (1957), would appear sufficient to prevent the setting up of an oviposition rhythm. Finally, the fact that the genetical control of this character is probably simple suggests a corresponding simplicity in its expression. It seems preferable for the present to accept the more obvious explanation with the proviso that subsequent experiments may make it necessary to review the situation.

There is clearly no direct genetical association between this aspect of laying habit and DDT-resistance, and the significance of the difference in behaviour between strains is still not understood.

Unconscious selection during many years of laboratory culture is well known as a common cause of differences between strains, and this explanation for the observed differences in the present investigation cannot be overlooked. Evidence is in fact contradictory: although the AS and NS strains, which tend to lay on the 'light side,' have the longest history of laboratory culture, the NS strain has been maintained throughout much of the 30 years of its laboratory life in total darkness. This would not seem conducive to the development of a 'light-laying' habit, indeed quite the opposite. Moreover, all strains except KS had been colonised for several years and all were thus well-established colonies. It is also possibly significant that one strain (KS), which was of recent origin and which showed a tendency to lay in dark situations, did so to a less marked degree than did two of the long-established (3-4 years) resistant strains. There would seem to be no correlation between 'dark laying' and the time during which a strain had been colonised.

The results from the experiment in which the STR strain was selected for 'dark laying' and 'light laying' might suggest that mild inbreeding could cause a reversion to 'light laying.' In each generation the numbers used approximated to 20 females and 20 males. However, since only a proportion of females would probably contribute to the next generation, the level of inbreeding would be greater than this. Assuming a single female to lay only at one oviposition site or the other, the mean effective number of females in each generation over seven generations of selection would have been STL:8 and STD:12. While it is suspected that a comparable level of inbreeding was commonly practised (for one or two generations at least) in stock cultures of this strain and no change in laying habit could be detected during the 18 months of the investigation, prolonged inbreeding at this level must remain a possible explanation.

The possibility that 'dark laying' may be a characteristic of var. *queenslandensis* has already been mentioned, but no definite conclusions can be drawn at this stage. There would seem, however, to be no obvious connection between laying habit and the geographical origin of the strain. Thus the NS and KS strains, both originating from West Africa, behaved quite differently, as did the AS and STR strains, both from the New World.

A further possibility is that 'dark laying', being associated at its highest level with a previous history of DDT treatment, might be an instance of behavioural resistance, a selective effect by DDT genetically independent of physiological resistance. While in the absence of direct evidence this must also remain a tentative explanation, it seems of interest that Surtees (private communication) has found that a West African strain of *A. aegypti* that showed a preference for particularly dark situations when ovipositing also rapidly developed an increased tolerance to DDT under laboratory selection pressure. This strain was characterised by a markedly flat regression line before selection, indicating that it was already heterogeneous in its response to DDT and suggesting the possibility of prior (field) exposure.

As to whether selection for the 'dark-laying' habit can be held responsible for the apparent increase in tree-hole breeding in Trinidad (and now also in Brazil (Pinto Severo, 1959)) or whether this has been caused merely by the destruction of more conventional breeding places, is still a matter of uncertainty. Studies on the breeding habits of DDT-treated and untreated populations under field conditions should be of great value in clarifying the situation.

Summary.

Seven strains of *Aedes aegypti* (L.), three DDT-resistant (from Trinidad (2) and Haiti) and four susceptible (from West Africa (2), Arabia and U.S.A.) have been investigated with respect to their attraction to oviposition sites illuminated at different levels. The resistant strains have tended to choose the darker sites (at 0.02 lumen per sq. ft. and below) and susceptible strains those illuminated at between 3.5 and 6.5 lumens per sq. ft.

Genetical crosses between a 'dark-laying' (resistant) strain and a 'light-laying' (susceptible) strain indicated that 'light laying' was fully dominant over 'dark laying' and it is suggested that the mechanism involved is probably simple. No linkage with the gene for physiological resistance to DDT could be demonstrated.

The expressivity of this character varied in certain circumstances with a tendency among most strains to lay at darker sites after the second and subsequent blood-meals. In spite of these variations, behaviour at any one time remained reasonably predictable.

The reflectance of the oviposition site did not appear to be important in determining the difference in behaviour between 'dark-laying' and 'light-laying' strains. On the contrary, it seemed probable that 'dark-laying' strains were attracted more to the dark situation in which the dimly-lit site was found rather

than to the site itself. There was some evidence of exploratory activity in the initial stages of oviposition by a 'dark-laying' strain.

Resting habits proved very similar in two strains differing markedly in oviposition habit. For both strains, 0.1 lumen per sq. ft. was apparently too low to be attractive and 12 lumens per sq. ft. too high. Resting behaviour did not appear to be under such critical control by light as was oviposition.

Possible explanations for the differences in 'oviposition light preference' are discussed.

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APPENDIX.

It is necessary to give an explanation of the method used to interpret the results from reciprocal crosses and back-crosses between strains differing in their 'oviposition light preference.'

In the example quoted, a strain laying 17.4 per cent. eggs in the dark (the 'light-laying' parent) was crossed with one laying 65.2 per cent. (the 'dark-laying' parent). The procedure for interpreting the results obtained was as follows:—If the tendency to lay a low percentage of eggs in the dark had been due to a single autosomal dominant factor, then, according to basic genetic principles, the percentage laid in the dark by the F_1 would have been 17.4 (as in the 'light-laying' parent) and by the F_2 $\frac{(3 \times 17.4) + (1 \times 65.2)}{4} = 29.3$ per cent. Actual (mean) percentage values obtained were, in fact, F_1 (AS ♀ × STR ♂) = 17.7 ± 7.2 , F_1 (STR ♀ × AS ♂) = 22.1 ± 7.2 and F_2 (AS ♀ × STR ♂) = 28.0 ± 7.2 and F_2 (STR ♀ × AS ♂) = 31.8 ± 7.2 , and thus there was very little margin of difference between observed and expected results.

Similarly in the back-cross to the 'dark-laying' parent, a percentage of $\frac{17.4 + 65.2}{2} = 41.3$ was expected, which agreed reasonably well with the experimental result of F_1 (AS ♀ × STR ♂) ♀ × STR ♂ = 45.8 ± 5.6 per cent.

This method of calculating expected values depends on there being no difference in fertility between parents and hybrids.

AN EXPERIMENT IN THE USE OF DISCRIMINATIVE CLEARING FOR THE CONTROL OF *GLOSSINA MORSITANS* WESTW. IN ANKOLE, UGANDA.

By J. M. B. HARLEY and R. D. PILSON *

*East African Trypanosomiasis Research Organization,
Tororo, Uganda.*

It has been known for many years that *Glossina morsitans* Westw. concentrates during the dry season in the woodland immediately surrounding open glades or valleys and that breeding at this time takes place largely in these areas (Jack, 1912; Shircore, 1914; Swynnerton, 1921). This led Shircore and Swynnerton to suggest that an attack on the concentration sites offered a possible method for eradication of the fly over wide areas at reduced cost. In the first attack of this kind against *G. morsitans*, this species disappeared from an area of *Brachystegia-Isoberlinia* woodland in Northern Rhodesia following the felling of the woody vegetation of the concentration sites, after several years of fire exclusion had shown little effect on the fly density (Glover & others, 1955). This type of clearing has since been widely applied in East Africa against this species of tsetse. Clearing of 10 per cent. or less of the infested areas has been followed by large reductions in, and sometimes disappearance of, the tsetse population (Bursell, 1955; Ford, 1954a; Lloyd, 1959). Clearing of this type has also proved effective against the closely related *G. swynnertoni* Aust. (Napier Bax, 1943).

Felling of the woody vegetation of the concentration sites is usually referred to as discriminative clearing. This follows the suggestion of Napier Bax (1943) that this term should be restricted to the removal throughout the area of a definite vegetation type made up of a number of species. He also suggested that the previously synonymous term, selective clearing, should be used for the removal of certain species of trees throughout the area concerned. The work described below is discriminative in that one definite vegetation type only was treated. However, it was considered unnecessary to remove *all* species of woody vegetation within this type, which was modified by the removal of certain species only.

In Ankole District, Uganda, *G. morsitans* occupies a vegetation community of wooded grassland with *Acacia* species. In 1949, Glover (unpublished) observed that the concentrations there were associated with a double-storey vegetation. This comprised an upper storey formed largely by *Acacia gerrardii* between 20 and 30 ft. tall, with a lower storey composed of *Acacia hockii* and thickets on termitaria. In the locality where he made these observations, in the range of hills to the south-west of Sanga (fig. 1), the concentration sites were in valleys but it was later found that, further north, they also occurred on top of small hills and on hill slopes. At the same time it was realised that the concentrations of *G. morsitans* in other parts of East Africa were also associated with double-storey vegetation, although this was composed of different tree species. However, concentrations in *Brachystegia-Isoberlinia* woodland occurred in the ecotone or vegetational interzone between the general woodland and the open seasonal swamps (*e.g.*, Swynnerton, 1936) in contrast to those in Ankole, which occurred in the only vegetation type including tall trees forming a fairly continuous canopy, these being surrounded by open grassland with scattered trees and thickets.

* Now at Department of Tsetse and Trypanosomiasis Control and Reclamation, Salisbury, Southern Rhodesia.

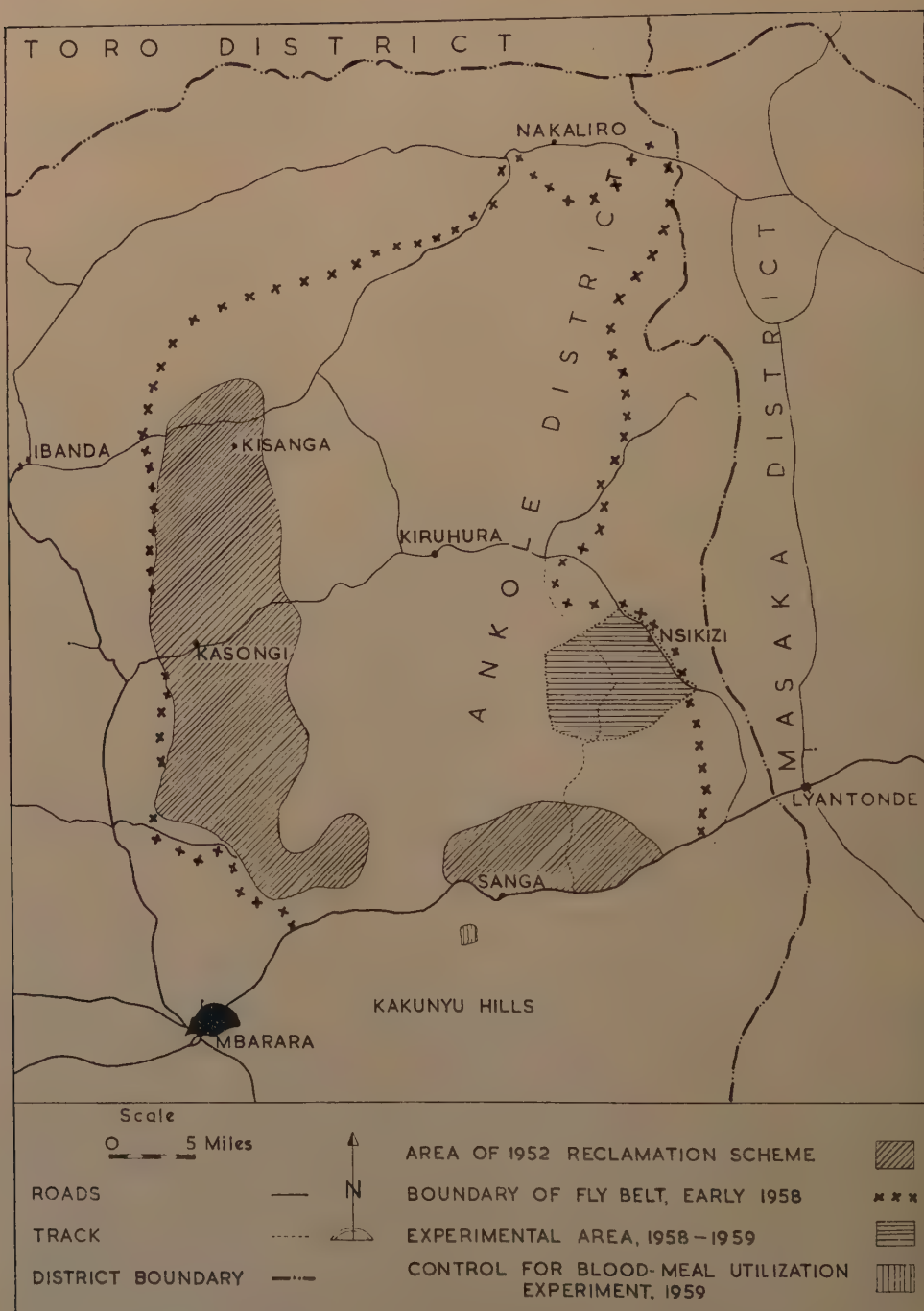


Fig. 1.—Map of the north-eastern area of Ankole District, Uganda, showing the size of the northern part of the fly-belt in 1952 at the start of the pilot reclamation scheme, the approximate perimeter early in 1958, as far as this was known, and the location of the discriminative clearing experiment 1958-59.

It has also been observed that flies caught in the concentrations in the *Brachystegia-Isobrerlinia* woodland were generally not hungry so that these areas did not represent feeding grounds (Swynnerton, 1936; Glover & others, 1955). Ford (unpublished) and later Harley (unpublished) observed that flies in the concentrations in the wooded grassland of Ankole also have a lower mean hunger stage (Jackson, 1933) and a lower proportion of females and teneral flies * than do samples from other parts of the fly-round. The concentrations in Ankole were present at all seasons but tended to be less pronounced during the rains.

In 1952, a pilot reclamation scheme (fig. 1) was started by the East African Tsetse & Trypanosomiasis Research & Reclamation Organization (EATTRRO) in Ankole District to find and apply a discriminative-clearing technique that would halt an advance of *G. morsitans* and eradicate it from an area of some 260 square miles of wooded grassland with *Acacia* species. It was considered that the removal of certain of the upper-storey trees, over 20 ft. tall, from the double-storey vegetation of the concentration sites described by Glover, would probably render this vegetation unsuitable and result in a general reduction in fly density. At first the results appeared promising and tsetse densities fell to a low level. For example, two fly-rounds at Kisanga (fig. 1), which were the first to be affected by the treatment, showed a 77 per cent. reduction, from a mean apparent density † of 25.1 for the third quarter of 1952, immediately before treatment, to 5.7 for the corresponding period in 1953 (Ford, 1954b). The mean apparent density decreased further to 0.25, corresponding to a reduction of 99 per cent. of the original value, for the period December 1955 to February 1956, rather more than three years after treatment (Ford, 1953, 1954b, 1955, 1956). However, later on and further south the effect on tsetse density was less marked. The fly-belt also extended both to the north and east of the pilot scheme area, to near the northern and eastern boundaries of Ankole District, and it became clear that in many areas more comprehensive clearing would be necessary to achieve eradication. Further clearing was carried out, culminating in late 1957 with the felling of all upper-storey trees of this double-storey vegetation type in a belt, between 6 and 14 miles wide, inside much of the eastern border of Ankole, in an attempt to confine the flies. It was intended subsequently to apply this degree of clearing to all areas in which tsetse remained, and the object of the present investigation was to determine whether this degree of clearing would result in eradication of *G. morsitans*.

Description of the experimental area.

The experimental area of 48½ sq. miles lies immediately west of Nsikizi (lat. 0° 18'S., long. 31° 3'E.), some 34 miles north-east of Mbarara, the headquarters of Ankole District (fig. 1). It is divided by a central track into the eastern or Nsikizi area of 28½ sq. miles and the western or Rwemondo area of 19½ sq. miles. The altitude is from about 4,400 to 4,700 feet.

The mean annual rainfall at Kiruhura, some 13 miles to the north-west, is 35.6 in. and at Lyantonde, some 13 miles to the south-east, is 37.2 in. but in 1958, which was a dry year, only 26.7 in. were recorded at Kiruhura. No figure is available from Lyantonde for 1958. It is normally dry from about late December to late February and from late May to early August, with wet seasons intervening, although the periods are variable and some rain is liable to fall each month. From March 1958 to August 1959 the recorded monthly mean maximum temperatures at Mbarara were between 78.0 and 81.4°F. and the mean minimum between 56.8 and 60.6°F. The climate is thus equable compared with that of many areas inhabited by *G. morsitans*.

* A teneral tsetse fly is one that has not yet taken its first blood-meal and has a characteristic soft feel.

† The apparent density is the number of non-teneral male flies caught per 10,000 yd. traversed.

TABLE I.
Vegetation of the experimental area.

Vegetation type	Upper storey	Lower storey	Grasses	Occurrence
1	<i>Acacia gerrardii</i> ; <i>A. sieberiana</i> var. <i>sieberiana</i> ; <i>A. sieberiana</i> var. <i>vermoesenii</i> ; or <i>Albizia</i> spp.	<i>Acacia hockii</i> with thickets of <i>Rhus</i> sp., <i>Grewia</i> sp., <i>Carissa</i> sp., <i>Scutia</i> sp. or <i>Capparis</i> sp.	<i>Brachiaria soluta</i> ; <i>Setaria longisetia</i> ; <i>Panicum</i> sp.; <i>Setaria</i> sp.	Widespread in 'pockets'—groups of two or more upper-storey trees, of which <i>A. gerrardii</i> is the most common representative
2	<i>Euphorbia candelabrum</i>	<i>Acacia hockii</i> with thickets of <i>Rhus</i> sp., <i>Grewia</i> sp., <i>Carissa</i> sp., <i>Scutia</i> sp., or <i>Capparis</i> sp.	<i>Brachiaria soluta</i> ; <i>Setaria longisetia</i>	In small 'pockets' or groups mainly on ridges but is not widespread
3	<i>Acacia gerrardii</i> ; <i>A. sieberiana</i> ; or <i>Albizia</i> spp.	<i>Acacia hockii</i>	<i>Themeda triandra</i> ; <i>Cymbopogon afronardus</i>	Mainly in the large drainage lines. The open aspect of the trees and the tall grass species immediately distinguishes this from type 1
4	None	<i>Acacia hockii</i> with thickets of <i>Rhus</i> sp., <i>Grewia</i> sp., <i>Carissa</i> sp., <i>Scutia</i> sp., or <i>Capparis</i> sp.	<i>Brachiaria soluta</i> ; <i>Setaria longisetia</i>	Throughout the area
5	None	<i>Acacia hockii</i> scrub.	<i>Themeda triandra</i> ; <i>Cymbopogon afronardus</i>	Common and widespread, mainly on hill-slopes and in narrow valleys
6	<i>Euphorbia candelabrum</i> (not always present)	Thickets of <i>Rhus</i> sp., <i>Grewia</i> sp., <i>Carissa</i> sp., <i>Scutia</i> sp. or <i>Capparis</i> sp.	<i>Brachiaria soluta</i> ; <i>Setaria longisetia</i>	Scattered throughout the area near water-holes or on termittaria
7	None	None	<i>Themeda triandra</i> ; <i>Cymbopogon afronardus</i>	Open grassland. Mainly on hill-tops. Small clumps of type-6 vegetation are scattered throughout
8 Cultivation	None	None	None	Found along the eastern and southern boundaries of experimental area

Note: The nomenclature of the *Acacia* species used here follows the recent re-classification of Brenan (1959). In previous reports and publications on Ankole, *A. gerrardii* was referred to as *A. hebecladoides*; *A. hockii* to *A. holstii*, *A. stenocarpa* or *A. seyal* var. *multijuga*; *A. sieberiana* var. *vermoesenii* to *A. vermoesenii*.

The common large mammals include buffalo (*Syncerus caffer*), eland (*Taurotragus oryx*), topi (*Damaliscus korrigum*), waterbuck (*Kobus defassa*), bushbuck (*Tragelaphus scriptus*), reedbuck (*Redunca redunca*), duiker (*Sylvicapra grimmia*), oribi (*Ourebia ourebi*), zebra (*Equus burchellii*) and wart-hog (*Phacochoerus aethiopicus*). Impala (*Aepyceros melampus*), bush-pig (*Potamochoerus porcus*), ant-bear (*Orycteropus afer*), porcupine (*Hystrix* sp.) and several carnivores also occur. Cattle occasionally enter the area. There are numerous water-holes and no large-scale migration of the fauna occurs. The local movements of several species are influenced by the fresh grass growth appearing in those places through which grass fires passed during the dry season.

The vegetation is simple and the number of woody species and grasses few. Eight vegetation types can be recognised and are described in Table I. All clearing referred to in this paper has been confined to the felling of the upper-storey trees of type-1 vegetation.

Discriminative clearing in the experimental area prior to 1958.

In mid-1955, all specimens of *Acacia gerrardii* over 20 ft. tall in vegetation type 1, where this was considered to form suitable concentration sites for *G. morsitans*, were felled over the whole experimental area. It was this régime that had been followed by considerable reduction in tsetse density in many of the areas previously treated. The apparent density on the only fly-round in this area at that time, Kamalia (fig. 3), fell from a mean of 37 for the six months before treatment to 2.3 for the three months following. Flies, however, did not disappear and, after a survey in April 1956, felling of all *A. gerrardii* was carried out in those remaining pockets of vegetation type 1 that were of more than five acres in extent and in which flies were found, irrespective of the heights of the trees. When Kamalia fly-round was re-opened in August of that year it was found that the apparent density had risen to 18; this gradually declined to about 12 over the following 18 months. Subsequently, in 1957, the majority of the remaining *A. gerrardii* in vegetation type 1 were felled in the Nsikizi area and east to the Ankole district border, irrespective of height of tree, size of pocket or occurrence of fly.

Thus, at the beginning of 1958, in the Rwemondo area and further to the west, much of the type-1 vegetation remained untouched while in the Nsikizi area and further to the east most of the vegetation of this type had been treated. From the subsequent estimates of the percentage of each area affected by discriminative clearing (see p. 567) it will be seen that, at this time, roughly two-thirds of this vegetation remained unmodified at Rwemondo and one-third at Nsikizi.

Preliminary investigation, 1958.

In February and March 1958, reconnaissance in the vicinity of Nsikizi showed that *G. morsitans* was still present, despite the intensive discriminative clearing carried out. It appeared that flies were rare in the east, near the road through Nsikizi, and more numerous towards the west. Four transect fly-rounds (Ford & others, 1959) were laid out, extending westwards from the road for about 2½ miles (fig. 2). These confirmed that there was a rising gradient in fly density from east to west, with concentrations near the western extremities. Reconnaissance in the Rwemondo area showed that relatively high fly densities occurred there.

The distribution of the flies suggested that the Nsikizi population was not permanent but was maintained by immigration from the Rwemondo area, and the recapture on the Nsikizi fly-rounds of flies that had been marked and released on the central track showed that immigration did occur.

There was no record of the fly density in the Nsikizi area prior to treatment. It was therefore unknown whether the relatively low fly density observed there early in 1958 was the result of the treatment, but as the adjacent Rwemondo area had supported a relatively high fly density for at least three years there was

clearly some difference that rendered the former less favourable to tsetse. Reconnaissance and such fly-round data as were available from the Rwemondo area showed that fly concentrations were associated with the remaining untreated type-1 vegetation. As most of this vegetation in the Nsikizi area had already been modified, it was considered that this was the probable cause of the difference in fly density between the two.

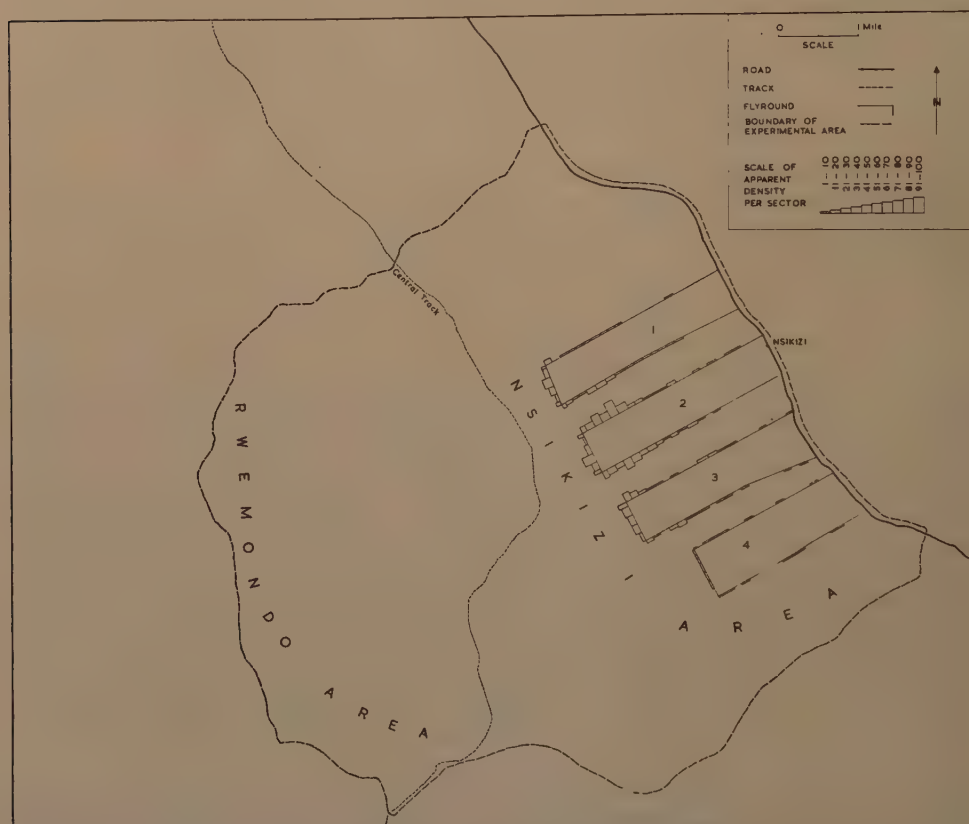


Fig. 2.—The distribution of non-teneral males of *G. morsitans*, March–May 1958, prior to the final phase of discriminative-clearing treatment. The heights of the rectangles erected on individual sectors of the fly-rounds represent the apparent densities.

Some association of fly with stands of tall specimens of *Acacia hockii* in vegetation types 2 and 4 (Table I), where these were adjacent to type-1 vegetation, was also observed. Such an association was known to have occurred on parts of Kamalia fly-round before the vegetation of this area was first treated in 1955. However, from the definite reduction in apparent density on this fly-round following this treatment (see p. 565), together with the marked tendency for subsequent concentrations to occur in the remaining type-1 vegetation and the complete absence of concentrations in tall stands of *A. hockii* where these were not adjacent to the type 1, it seemed unlikely that *A. hockii* would support a permanent tsetse population after the type 1 had been made uninhabitable.

Experimental investigations, 1958-59.

In order to test whether the removal of all upper-storey trees, irrespective of species or height, of all type-1 vegetation would result in the eradication of *G. morsitans*, it was decided to apply this degree of clearing first to the Rwemondo area and, subsequently, to complete the treatment of the Nsikizi area by the removal of the remaining upper-storey trees. Complete eradication was not expected as the area was not isolated but it was considered that a westward shift to the Rwemondo area of the population gradient previously observed near Nsikizi (fig. 2) and a marked over-all reduction in apparent density would prove the success of the method. The appearance of an east-west population gradient in the Rwemondo area would also indicate the modification of the vegetation as the responsible factor.

Clearing treatments applied.

The Rwemondo area was discriminatively cleared between May and October 1958, and the Nsikizi area between October 1958 and April 1959. The area of the numerous, and mainly small, pockets of type-1 vegetation treated was not easily measured; 2,123 separate pockets have been treated in the Rwemondo area and 1,855 in the Nsikizi area since 1955. The total area treated was calculated both from the number of trees felled and also by measuring the size of the pockets, plotted on aerial photographs and subsequently enlarged, with a planimeter. Counts in three sample localities had shown upper-storey trees to occur in the experimental area at a density of approximately 150 per acre, and the total number of trees felled was divided by this figure to give an estimate of the acreage treated. From these data it is estimated that approximately 7.5 per cent. of the Rwemondo area was treated during this final phase of the discriminative clearing, making a total of 11 per cent. treated since 1955. The corresponding figures for the Nsikizi area are approximately 2.3 and 7 per cent.

Methods of studying fly populations.

Transect fly-rounds were used to determine the distribution and density of the tsetse population. Four rounds had been laid out early in 1958 for the preliminary investigation and, towards the end of May 1958, at about the time clearing commenced, a further three were started in the Nsikizi area, three more in the Rwemondo area, where, in addition, the old fly-round, Kamalia, was re-opened, and a control fly-round was laid out to the north-west of the experimental area; a second control fly-round was laid out in August. These fly-rounds (fig. 3) were between four and six miles long and were divided into sectors of 200 yd., except at Kamalia where the sectors were 100 yd. long. For the first few months one to three traverses of each were made weekly except for fly-round 2 in the Rwemondo area where four traverses were made weekly. From the end of September 1958, each fly-round was traversed twice weekly. Apparent density, mean hunger stage and the percentages of female and teneral flies among the catch were calculated monthly for each fly-round. Concentrations were regarded as occurring on those fly-round sectors on which the catch exceeded the expectation on a Poisson series, as described by Ford & others (1959).

Flies of both sexes containing visible blood were killed and the blood expressed onto filter paper which was sent to the Lister Institute of Preventive Medicine for determination of the host species. Searches for resting flies, mainly aimed at collecting gorged flies, were carried out in the control area.

Flies were caught twice weekly off a vehicle stopping at half-mile intervals along the central track, individually marked (Jackson, 1953) and released. Those caught on fly-round 2 in the Rwemondo area were similarly marked and released. All other flies were killed.

Results.

The apparent densities of *G. morsitans* for each of the fly-rounds are given in Table II, which includes data to the end of August 1959, when work ceased. The effect of the treatment differs between fly-rounds: on some there was a rise during the operations, possibly due to disturbance, while the post-treatment density was variously higher, lower or virtually the same as that obtaining previously. In no case did the expected marked reduction follow clearing, but there was a less general rise in apparent density in the experimental area than was shown by the controls. No marked increases were observed in values of the mean hunger stage, in the



Fig. 3.—The distribution of non-tenatal males of *G. morsitans*, June–October 1958, during the treatment of the Rwemondo area.

proportions of female or teneral flies, or in the proportion of males attacking the catching party—increases that would have been indicative of greater stresses acting on the population.

The distribution of the male catch during the two treatment periods and the post-treatment period is illustrated in figs. 3 to 5. The density gradient originally observed near Nsikizi (fig. 2) showed no marked change, neither was there any indication of an east–west gradient being formed in the Rwemondo area.

Following the final phase of discriminative clearing, all fly concentrations occurred within, or close to, groups of *Acacia hockii* trees about 12 to 18 ft. tall within vegetation types 2 and 4. Also, in all localities where such vegetation was present, fly concentrations were observed regularly in most months. Groups of *A. hockii* of this height form a more or less continuous canopy which, associated with thicket species and short grass, apparently affords suitable conditions to

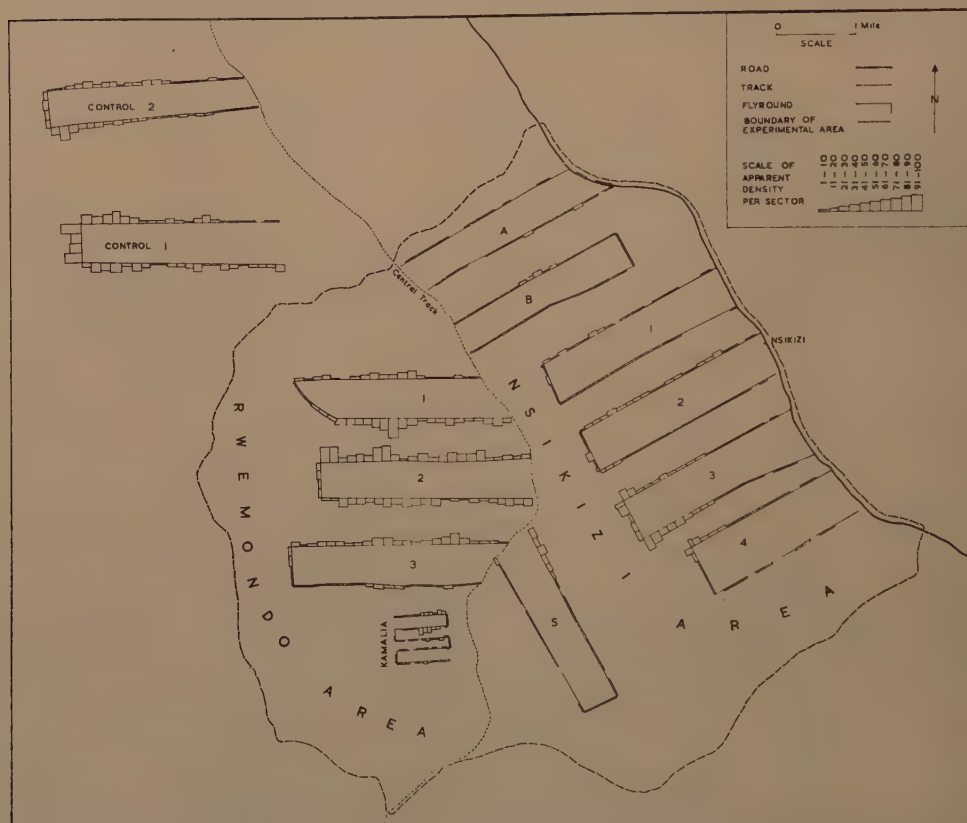


Fig. 4.—The distribution of non-teneral males of *G. morsitans*, October 1958–April 1959, during the treatment of the Nsikizi area.

maintain the population at unreduced density. In many instances the concentrations were adjacent to the treated type-1 vegetation but in other localities new concentrations have appeared. In the Nsikizi area, these concentrations occurred close to the western extremities of fly-rounds 1 to 4, and were apparently not dependent on immigration from the Rwemondo area, although immigration of marked flies still took place. Concentration sites were mainly on ridge-tops and at the heads of valleys, but also occurred on hill-slopes. Large mammals, particularly buffalo, frequented many of these sites during the day, while several ridge-top sites were in close proximity to game paths.

The composition of the catch in concentrations differs from that on other sectors of the fly-rounds; results from three of the fly-rounds during the post-treatment period are given in Table III. In the concentrations, the mean hunger

stage and other measures (percentage of non-teneral males caught on the party and the percentage of females and teneral) that are generally regarded as indicative of the hunger of the population, are appreciably lower. Thus, despite the association of concentrations with game, which was particularly noticeable on Rwemondo fly-rounds 1 and 2, it is clear that these localities are not feeding grounds.

The identification of blood-meals of gorged flies collected (a) before the final phase of the discriminative clearing started and (b) during, and immediately after, the treatment of the Rwemondo area, is given in Table IV. The difference between



Fig. 5.—The distribution of non-teneral males of *G. morsitans*, May-August 1959, the post-treatment period.

samples (a) and (b) from the control area, as regards the number of flies feeding on cattle, is probably the result of an increase that took place in the numbers of cattle grazing in this area towards the end of 1958. The difference between samples (a) and (b) from the Nsikizi area and also between those from the control area as regards the number of feeds on buffalo, are probably the result of the extensive local movements undertaken by these animals; if they were the result of disturbance due to the presence of the labour gangs, the Rwemondo samples would be expected to show an even greater difference, but this is not the case. Thus it appears that the final phase of discriminative clearing had no significant effect on the feeding habits of the fly. There can also have been little disturbance

TABLE III.

Composition of catches of *G. morsitans*: comparison between concentrations and other sectors on three fly-rounds during the period May–August 1959.

	Rwemondo 1		Rwemondo 2		Nsikizi 3	
	Concentra- tions	Other sectors	Concentra- tions	Other sectors	Concentra- tions	Other sectors
Apparent density ..	108	22	120	39	86	8
Mean hunger stage ..	3.23	3.36	3.20	3.28	3.30	3.42
% NT males on party	14.8	37.3	13.9	24.9	19.4	30.1
% NT female	2.7	15.3	2.7	9.2	5.4	17.0
% Teneral	5.4	12.1	5.5	9.8	6.2	15.9
No. of sectors	6	39	9	41	8	41

% NT males on party: the number of non-teneral males caught on the fly-boy party, expressed as a percentage of the total catch of non-teneral males, the balance being taken on the ground and on vegetation.

% NT female: the percentage of females in the total catch of non-teneral flies.

% Teneral: the percentage of teneral flies in the total catch.

TABLE IV.

The source of food of *G. morsitans* in experimental and control areas.

		Rwemondo		Nsikizi		Control	
		a	b	a	b	a	b
Primates	Man	2	2	5	10	—	0.9
Suids	Wart-hog	43	46	15	24	28	32
	Bush-pig	—	—	—	1	—	0.9
	Unidentified	28	16	10	19	3	15
Bovids	Cattle	—	—	—	—	3	15
	Buffalo	21	19	35	15	32	9
	Bushbuck	—	0.4	10	1	3	6
	Duiker	—	1	—	1	—	—
	Eland	4	10	—	9	2	6
	Reedbuck	—	0.8	10	4	—	0.9
	Unidentified	—	4	15	14	18	14
Other mammals	Feline	2	—	—	—	—	—
	Canine	—	0.4	—	1	2	—
	Ant-bear	—	0.4	—	—	2	0.9
	Porcupine	—	—	—	1	—	—
	Unidentified	—	—	—	—	7	—
Totals		100	100	100	100	100	100.6
Number in sample ..		47	264	20	80	57	107

The figures are percentages of the total number examined in each set of samples.

Samples (a) were collected between March and May 1958, before the final phase of discriminative clearing.

Samples (b) were collected between June and December 1958, during and after the clearing of the Rwemondo area.

of the fauna by the labour gangs, an effect that had been thought might contribute to any reduction in the fly population. Wart-hog and buffalo were the predominant hosts.

Although the discriminative clearing carried out since 1955 had little apparent effect on the tsetse population, it seemed possible that it might have resulted in an increase in the general level of activity and therefore in an increase in the rate of utilisation of reserves together with a decrease in the length of the hunger cycle. Such an effect could have passed unnoticed by routine sampling techniques as hosts were numerous, offering the opportunity of frequent feeding, and the equable climate exerts little seasonal stress on the population. An experiment was therefore undertaken at the end of July 1959, during the dry season, to determine whether the rate of utilisation of the blood-meal of flies in the Rwemondo area differed from that of flies in a control area to the south of Sanga (fig. 1) where no clearing had been applied.

Flies in hunger-stage 4 were caught on a motor-vehicle, weighed on a torsion balance, fed, re-weighed, individually marked and released. This procedure was carried out on alternate days in each area between 0930 and 1330 hr. Searches for the released flies were made each day between the same hours. On recapture, flies were immediately killed with potassium cyanide and the thoracic size was determined (Bursell, 1960) on return to camp. The amount of chloroform-soluble substances (hereafter referred to as fat) and the residual dry weight (*i.e.*, the fat-free and water-free weight) of thorax and abdomen were determined on return to the laboratory by methods described by Bursell (1959). Data from the abdomens only were used in the calculation as the paint used on the thorax to mark the flies complicates the determination of the true weights.

Forty typical hungry flies were collected, kept singly in tubes and starved to death at high humidity. Their size, fat content, and residual dry weight were subsequently determined. A regression of the residual dry weight of the starved abdomen on size was calculated, from which the residual dry weight of the starved abdomen of individual experimental flies of known size could be deduced. The difference between this weight and the actual residual dry weight of the experimental flies was therefore the weight of the blood-meal residue remaining in the abdomen. This was expressed as a percentage of the solid matter in the blood-meal, which comprises 21.1 per cent. of the weight of the meal (Hammarsten, 1911). As the mean interval between release and recapture was slightly different for the samples from the two areas, the results from Sanga were corrected for the intervals experienced by the Rwemondo flies. The results are given in Table V.

TABLE V.

The residual blood-meal in abdomens of *G. morsitans* recaptured at different intervals after feeding (expressed as a percentage of the solids in the original meal).

				No. of days after feeding				
				1	2	3	4	5
Rwemondo	<i>n</i>	6	11	8	4	0
	Mean residual	56.13	20.83	13.63	7.35	—
	blood-meal %	± 8.04	± 2.21	± 2.70	± 0.84	
Sanga	<i>n</i>	10	20	12	6	3
	Mean residual	67.91	24.01	13.25	11.97	6.17
	blood-meal %	± 4.14	± 2.31	± 1.00	± 1.42	± 0.96

n=number in sample.

There is no significant difference in the percentage of blood-meal residue between the two areas except on day 4, when Student's t test for a comparison of means shows the difference to be just significant ($P < 0.05, > 0.02$). The mean temperature recorded at each release point by a thermohygrograph in a Stevenson screen for a week during the experiment was greater at Rwemondo (21.7°C. (71.1°F.)) than at Sanga (20.9°C. (69.6°F.)), and may account for the slight differences between the samples. This difference in mean temperature is, however, not necessarily the result of clearing and may be due to topography; the Sanga release point was in a valley where the daily variation of temperature and humidity was considerably greater than at the Rwemondo release point, which was on top of a ridge.

An attempt to establish whether there was any difference in the fat cycle of the flies released in the two areas failed owing to considerable variation in fat content both of the recaptured flies and of control samples killed on first capture (cf. Bursell, 1959; Jack, 1939).

Discussion and conclusions.

The failure of the final phase of the discriminative clearing to have any marked effect on the population of *G. morsitans* is disappointing when similar, though less comprehensive, clearing in 1952 and 1953 was followed by a considerable decline in population density at Kisanga (p. 563), an area of similar vegetation and climate some 25 miles distant. The essential difference between the two areas would seem to lie in the presence in eastern Ankole, including the experimental area, of a taller growth of *Acacia hockii* in vegetation types 2 and 4 (i.e., trees about 12–18 ft. high, see p. 570), which proved adequate to support the tsetse population at unreduced density after the double-storey vegetation of the primary concentration areas had been modified.

It appears that before substantial reduction of the tsetse population can be achieved the groups of taller specimens of *A. hockii* must be removed in addition to the upper-storey species of the type-1 vegetation. It is considered that a practical policy for clearing this area would be to fell the majority of specimens of *Acacia* and *Albizia* species over about eight ft. tall. Such a policy would ensure that there was no possibility of concentration sites being formed by the growth of those trees only slightly less than 12 ft. tall before reinvasion could be prevented by adequate measures in the neighbouring areas. This type of clearing would be selective rather than discriminative. It would be more easily applied as it would not necessitate the location of the numerous, small concentration sites on the ground by skilled staff, to which the labourers have to be guided. It would, however, affect considerably more than the 10 per cent. of the infested area that has for some time been generally considered adequate to control *G. morsitans* in *Brachystegia-Isobertinia* woodland in eastern Africa. A rather similar policy was advocated by van den Berghe & Lambrecht (1956) for part of Ruanda Urundi where this species of *Glossina* occurs in a similar vegetation community, although they considered that it was unnecessary to fell *Acacia hockii* or *Albizia* species, but only *Acacia* trees over four metres tall.

Summary.

The situation following different degrees of discriminative clearing against *G. morsitans* Westw. was examined in eastern Ankole District, Uganda. In one area, where the treatment had been more intensive, the fly density was low, and it seemed that the population might be maintained by immigration from an adjacent area to the west, where treatment had been less intensive and in parts of which fly density was relatively high. The application of more intensive discriminative clearing of the fly's concentration sites to the western area, together with completion of the treatment of the eastern, failed to result in any marked effect on the

tsetse population except as regards its distribution. Before this intensive clearing, concentrations of *G. morsitans* occurred in a double-storey vegetation type, the chief component of the upper storey of which consisted of *Acacia gerrardii*. After the upper-storey trees in this were felled, concentrations were found in groups of tall *Acacia hockii*, 12–18 ft. high, which had appeared to be of little importance to the fly before clearing. A year after the treatment of the western area there was no apparent reduction in fly density. The feeding habits and rate of utilisation of reserves of the flies showed no significant change as a result of clearing.

It seems that the failure of the discriminative clearing régime applied in this area, compared with the effect produced by less intensive clearing elsewhere in Ankole, is due to the presence in this area alone of the groups of tall *A. hockii*.

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STUDIES ON THE BIOLOGY AND CONTROL OF *LACHNOSTERNA*
CONSANGUINEA (BLANCH.), A PEST OF SUGARCANE
 IN BIHAR (INDIA).

By A. N. KALRA and J. P. KULSHRESHTHA

E.M.

Indian Institute of Sugarcane Research, Lucknow.

Larvae of Lamellicorn beetles belonging to the MELOLONTIDAE and related families have been known to cause extensive damage to sugarcane in different countries. In Queensland, damage by larvae of *Dermolepida albohirtum* (Waterh.) to cane plantations was reported to be widespread in the early thirties (Bell, 1935). In Tanganyika (Jepson, 1956), damage by Lamellicorn larvae, chiefly of *Cochliotus melolonthoides* (Gerst.) (MELOLONTIDAE), occurred year after year and many cane estates were rendered temporarily unfit for cane cultivation. In India, although species of white grub were reported earlier (Lefroy, 1909, p. 254; Ghosh, 1937) from sugarcane, they were not known to cause damage to the crop until August 1956, when a species later identified as *Lachnosterna consanguinea* (Blanch.) (Gupta & Avasthy, 1957a, b) appeared in very large numbers at Dalmianagar, on the left bank of the river Sone in the Shahabad district of Bihar. The pest inflicted heavy losses to cane growers in the very first year of its appearance, and in some of the infested fields as much as 80 per cent. of the crop dried up and was rendered unfit for crushing. All attempts to control the pest in the infested standing crop by application of insecticides proved of no avail.

Preliminary studies carried out at Dalmianagar during 1956-57 (Gupta & Avasthy, 1957a, b) threw some light on the biology of the pest and possible means for its control. For further study of the problem, a scheme was sanctioned in September 1957 by the Ministry of Food and Agriculture, Government of India, under the Indian Institute of Sugarcane Research, Lucknow. Under this scheme, initial studies on the extent and nature of damage, utility of light-traps for destruction of adults, and the use of certain insecticides for control of larvae in the soil were conducted by B. D. Gupta & G. N. Rao (unpublished). Further studies on the biology and control of the pest were continued by the authors, and in this paper an account of this work is presented.

Distribution of the pest.

Gupta & Avasthy (1957b) have recorded attack by the pest on sugarcane in some of the villages around Dalmianagar town and in some areas near Tilothu town, about 10 miles up-stream on the Sone. The pest has since been recorded doing damage to cane in Mednipur, Kanchanpur, Suara, Sakhra and Kuakoch in Shahabad district and also in such widely separated areas as Jineshwargarh (Shahabad) and Bihta in Patna district. The presence of the pest in the latter areas raises grave apprehensions about its occurrence in other cane-growing tracts of Bihar, particularly those situated along the river Sone comprising light sandy soils, which are most suitable for development and activity of the larvae.

Nature and extent of damage.

Gupta & Avasthy (1957b) have described the nature and extent of damage by the pest. As stated by them "the grubs feed on the rootlets and root-hairs of sugarcane in such a way that the entire root system gets paralysed." The young

larvae, after hatching from the eggs, feed initially on tender roots of grass and then move to cane and are to be found associated with the main roots. They feed by cutting the side roots at their bases. Damage by the pest is more severe in the February-planted crop than in the October-planted crop, because in the latter the root system is better developed by the time attack of the larvae starts. The larvae were observed to injure the main stalk also, but were never found within the stalk. Once the larvae attach themselves to the roots of a clump, they remain confined to that clump for the rest of their feeding life, moving to a neighbouring clump only in the event of the original clump drying up. The highest population of larvae was reached in September.

Lightly infested clumps, in which the roots are not completely cut, show signs of recovery in October, at which time the larvae leave the roots and move deeper into the soil for pupation. But heavily damaged clumps dry out completely by the end of September or the beginning of October. Cane so damaged is a total loss.

A remarkable feature of damage by *L. consanguinea* has been that the infestation seems to occur only in light sandy-loam soils. Crops on clay soils are not affected. This is probably because light soils allow the gravid females better access for egg-laying and also afford easier movement of the larvae in the soil. Since the cane-growing belt along the Sone mostly comprises light sandy-loam soils, the pest constitutes a potential danger to cane growers in the area.

Seasonal history.

Overwintering adults (see p. 580) are stimulated to activity from April to June, when they emerge from the soil. A rush of emergence occurs when there is a shower of rain during this period. Eggs are laid during June-July when the soil has been moistened by the first showers of rain. From July to September, the grubs feed on the roots of cane or of grasses. The maximum damage to crop is done from the middle of August to the end of September when the grubs are fully grown. Pupation starts by the middle of September and continues till the end of October. At this time the grubs are at a depth of 0.3 to 1.5 m. in the soil. Adult emergence from pupae starts by the last week of September and by the end of November it is complete. The adults remain inactive within their earthen cells till the following April when they begin to leave the soil. The pest has only one brood in a year.

Biology.

Emergence of adults from the soil.

Sporadic emergence of adults from the soil usually starts in the last week of April when the maximum temperature ranges from 32.2 to 39°C. and the mean relative humidity between 24 to 33 per cent. The time of the rush of emergence of adults is mainly determined by the occurrence of the first showers of rains. Subsequent showers do not have much effect on emergence of the beetles unless the first shower is followed by a long dry spell, in which case there is again a rush of emergence soon after the second shower. However, it was noticed that the number of beetles emerging after the first shower of rain was much higher than that emerging after subsequent showers.

The adults usually emerge out of the soil in the evenings between 6 and 7 p.m. by making a circular hole 10 to 12 mm. in diameter. Gupta & Avasthy (1957b) have noted some emergence in the morning hours also, from 3 to 7 a.m. Holes made by the emerging beetles remain intact for some time; infested fields are studded with such holes soon after the first shower of rain.

After emergence, the beetles remain on the soil surface for a few minutes before flying to nearby trees or bushes, where they feed on the leaves. They remain active during the night and hide in the soil during the day. Loose sandy soil

underneath the trees or bushes is preferred for hiding or resting. The beetles do not go deeper than 5 to 15 cm. Oviposition takes place when they are hiding in the soil during the day-time.

Mating.

Although the emergence of adults, both males and females, starts in the last week of April, mating does not take place till the first showers of rain have fallen. The proportion of males is higher in the initial stages but later on more females emerge and by July the latter predominate. Mating pairs are frequently observed, soon after the first showers of rain, on bushes as well as on the ground between 6.30 and 7.30 p.m. During copulation the male assumes a more or less perpendicular position upon the female and inserts its curved and extended aedeagus into the protruded bursa copulatrix of the female. Mating lasts for about 5 to 20 minutes and takes place quite often during the life of both males and females.

Oviposition.

Development of the eggs starts soon after mating has taken place. The eggs are discernible through the abdominal sternites of the gravid female about 72 hours after the first copulation. The period between mating and oviposition was observed to be five days in the laboratory. Only two to three eggs were laid, singly, at a depth of 5 to 10 cm: in the soil by an individual female during the night. Gupta & Avasthy (1957b) have recorded egg-laying to a depth of 15 cm. and according to them as many as six eggs (arranged vertically in a row) were laid by one female at one time. Oviposition continues throughout adult life and as many as 50 to 60 eggs may be laid by a single female. The incubation period varied from 8 to 10 days in the laboratory in July at the maximum temperature of 28 to 38°C. and minimum temperature ranging between 24 to 30°C. with mean relative humidity varying from 60 to 98 per cent. As stated earlier, the females show a marked degree of selectivity in regard to soil, and moist sandy soils, which allow easy penetration, are preferred for egg-laying. Moreover, soils having some plant cover are preferred to fallow fields, though, in years of heavy infestation, eggs are laid in the latter types of fields also. It was further observed that oviposition was greater in fields having autumn-planted cane than in those with a spring-planted crop.

Feeding by the larvae.

After a three-hour resting period following hatching, the young larva becomes active and moves about in the soil in search of food. On coming across some tender roots (of grass, sugarcane, etc.) it starts feeding. In cane fields, the young larva feeds for a few days on grass roots and later moves to the cane roots 10 to 20 cm. below the soil surface. The growth of the larva depends upon the kind of plant on which it feeds. Those feeding on sugarcane roots grow more quickly and are full grown by the middle of August, when they measure 35 to 45 mm. in length and 7 to 10 mm. across the thorax, while those feeding on grass roots in fallow fields are only 20 to 25 mm. in length and 5 to 7 mm. across the thorax by that time. The larval period varies, therefore, from 8 to 10 weeks (depending on the kind of food) between July and September when the maximum temperature usually ranges between 28 and 39°C. and mean relative humidity is 65 to 96 per cent.

Gupta & Avasthy (1957b) observed some movements of larvae downwards and upwards in the soil in the morning and evening hours caused by "unfavourable soil conditions like flooding or the lowering of the temperature"; the present authors did not find any such movements except in the root system of the same clump. Rain or irrigation water had little effect on the larvae and they remained

attached to the cane roots. The major movement of the larvae takes place towards the end of September and beginning of October when they are full grown and have a tendency to move deeper into the soil for pupation.

As observed by G. N. Rao (unpublished), a high degree of cannibalism occurred among the larvae when these were placed together in a jar. For study of their life-history, habits, etc., the larvae had to be reared individually in separate pots.

Pupation and emergence of the adults.

The fully grown larva moves down, about the end of September or beginning of October, to a depth of 0.3 to 1.5 m. below the soil surface and makes an earthen cell there for pupation. This cell measures about 35 mm. in length and 15 mm. in diameter with round ends. The larva, which turns whitish with a yellowish tinge at this stage, rests in a semi-circular position for two to three days before finally turning into a pupa.

The adult emerges from the pupal skin by rupturing it on the ventral side, and it takes several hours to extricate itself out of the pupal skin. In the laboratory, the pupal period varied from 12 to 16 days during October–November, at a maximum temperature of 27 to 32°C. and mean relative humidity varying from 59 to 93 per cent. By the end of November, emergence of the adults is complete, but they remain inactive and confined to the pupal cells till the following April when emergence from the soil starts.

Length of life of adults.

Under cage conditions, beetles lived for 80 to 93 days, and there was no marked difference in this respect between the sexes.

Food-plants of the adults.

As stated above, the adults are active at night. Gupta & Avasthy (1957b) have recorded them as feeding on leaves of *Ficus glomerata*, *F. religiosa*, *Mangifera indica*, *Melia azadirachta* and *Zizyphus jujuba*. Besides these, the authors found the beetles eating leaves of *Terminalia arjuna*, for which the pest showed a marked preference, and also on *Rosa damascena*. So far no damage has been recorded on sugarcane leaves.

Attraction to light.

The beetles are attracted to light in large numbers during the early hours of the night, particularly between 7.30 to 9.30 p.m. However, when the adults are feeding they do not seem to be attracted to light even when a 300-c.p. Petromax lamp was brought close to them (Gupta & Avasthy, 1957b). Adult activity was more pronounced when the nights were dark, warm and humid. Very few beetles were observed to be attracted to light on rainy and windy nights. During the peak period of adult emergence, females formed about 40 per cent. of the light-trap collections, but gravid females were rarely taken at light-traps.

Flight range.

An attempt was made, in June–July 1959, to study the flight range of adults by releasing previously marked individuals at measured distances from lights and then sorting out such adults from collections made at the lights. These studies showed that, during calm nights, adults could fly to light for distances up to about 183 m. (600 ft.).

The adults when physically disturbed drop to the ground and crawl a little distance before taking wing again.

Description of the various stages.

The egg.

Gupta & Avasthy (1957b) have described freshly laid eggs as "milky white in colour and elongated in shape measuring about 3.0 mm. in length and 1.4 mm. in width." As the development of the embryo proceeds, the width of the egg increases to about 2.3 mm. in five to six days; the egg thus becomes almost spherical in shape. By the seventh day the egg assumes a dirty white colour and a brown comma-shaped mark appears on one side of it. This mark, which indicates the position of the mandibles of the developing larva, becomes more distinct on the eighth day. Finally the young larva emerges by rupturing the egg-shell in a zigzag manner.

The larva.

Just after hatching, the young larva is milky white in colour with shining brown tips of the mandibles. It measures 5.5 mm. in length. It remains inactive for about three hours after hatching; during this time, its body expands to about 9.00 mm. in length and assumes a cream colour, the legs and head becoming yellow. Fully grown larvae measure 35 to 45 mm. in length and 7 to 10 mm. across the thorax (see p. 579). According to Gupta & Avasthy (1957b), "a full grown grub is a white, fleshy and curved bodied insect, about 53 mm. long and 7 mm. broad at the thorax with brown head and three pairs of prominent legs. The abdominal segments being translucent, intestinal contents are clearly visible from outside. Two parallel rows of spines on the ventral surface of the terminal abdominal segment differentiate it from other white grubs belonging to family Scarabaeidae."

The pupa.

The freshly formed pupa is cream white in colour but gradually it changes to straw colour and ultimately becomes brown. The pupa measures from 20 to 23 mm. in length and 8 to 10 mm. across the thorax, depending upon the size of the larva from which it was derived. As the pupal stage advances, the head, antennae and legs turn brownish, the eyes dark brown and the elytra yellowish brown.

The adult.

The adult, on emergence from the pupal skin, has thin, crumpled, pinkish, papery elytra and a soft creamy-yellow abdomen. Later, the elytra become hard and stiff, and their colour changes to light brown and ultimately to dark brown. Males measure 16 to 22 mm. in length and 8.5 to 12 mm. across the thorax; females are 16.5 to 22 mm. in length and 9 to 12 mm. in breadth. Individuals of the two sexes are alike in outward appearance. However, in the male, the two bunches of round testes can be seen through the semi-transparent abdominal sternites, whilst in the gravid female eggs are discernible in the same way (figs. 1, 2); the abdomen of the female becomes a little distended also.

Control measures.

Gupta & Avasthy (1957b) have briefly described the various methods tried by them for control of this pest at the Bank Farm of Messrs. Rohtas Industries Ltd., Dalmianagar. Among these methods, collection of beetles at light-traps, hand-picking of the grubs and use of certain insecticides, viz., BHC dust, γ BHC emulsion, paradichlorobenzene, heptachlor and D-D Soil fumigant (a mixture of 1,3-dichloropropene and 1,2-dichloropropane) against the grubs, deserve special mention. They found, however, that all these methods failed to give a satisfactory control. Further studies were therefore conducted to find out some effective control measures both against adults and grubs.

(a) Control of the adults.

(i) *Collection of adults from food-plants*.—It was observed that the beetles could be collected in large numbers by vigorously shaking the trees or bushes on which they feed during the night. This operation could be most conveniently done between 7 and 9 p.m. The beetles that fell to the ground were immediately collected by hand and put into vessels containing water with a surface film of kerosene. During the peak periods of emergence, two labourers could collect as many as about 3,000 adults in two hours.



Fig. 1.—Ventral side of the abdomen of *Lachnosterna consanguinea*, male, $\times 4$.



Fig. 2.—Ventral side of the abdomen of *L. consanguinea*, female, $\times 4$.

This method of destruction of beetles had a distinct advantage over the light-traps because both males and females (including the gravid ones) were destroyed in almost equally large numbers.

(ii) *Control by insecticides*.—A non-replicated exploratory trial with BHC and DDT in 0.25 and 0.5 per cent. suspensions was laid out in June 1959 to test their efficacy when applied to bushes of *Z. jujuba* in cages. The bushes were sprayed in the evening and ten beetles were released in each cage immediately afterwards. One bush was left untreated as a control. Mortality among the beetles was recorded at 24-hour intervals till 96 hours after spraying.

As is shown in Table I, 0.5 per cent. DDT gave the best results.

TABLE I.

Comparative efficacy of sprays containing suspensions of DDT or BHC against adults of *L. consanguinea*.

Treatment	Mortality (%)—hours after spraying			
	24	48	72	96
DDT 0.25 per cent. ..	60	60	90	90
DDT 0.5 "	30	90	100	100
BHC 0.25 "	40	90	90	90
BHC 0.5 "	30	90	90	90
Control	Nil	10	10	10

(b) *Control of the larvae.*

Mechanical methods having earlier shown little promise against the larvae, their control by insecticides was studied further. It had previously been observed that application of insecticides in August–September, when the pest attack was at its maximum, proved ineffective, because by then the larvae were fully developed and could stand even high doses of insecticides. Application of insecticides was therefore made earlier in the season, *i.e.*, before the hatching of the eggs or when the larvae were yet young.

(i) *Application of insecticides in dusts.*—Two replicated experiments were laid out by B. D. Gupta & G. N. Rao (unpublished) in 1958. Aldrin, dieldrin, BHC, Chlordane and heptachlor were tried. In one experiment they were applied at the time of planting of the cane in February; in the other, they were applied in May at the time of the rush of emergence of beetles. A dust containing 10 per cent. BHC applied at the rate of 2 cwt. (22.4 lb. actual BHC) per acre at the time of planting, brought about a high mortality among larvae, but it had an adverse effect on the germination of the crop. The same dose of BHC applied at the time of the rush of emergence of adults in the second experiment gave better results in that it did not adversely affect germination.

Further trials were carried out in 1959 to study the possibility of reducing the dosage and to compare the efficacy of a single application with that of two. BHC, aldrin, Chlordane and DDT were applied as dusts at two dosage rates, *viz.*, 5 lb. and 10 lb. of the actual insecticide per acre. The dusts were applied uniformly over the soil surface in between the rows, using a perforated empty cigarette tin, and then worked into the soil. The experiment was laid out in two blocks with the variety Co. 617. In one block, the treatments were applied once only at the end of May (at the time of the rush of emergence of beetles), while in the other a second application was made in July also. The plot size was 1/40 acre replicated four times in each of the two blocks. The results of sampling in September, calculated per acre, are summarised in Table II.

TABLE II.

Efficacy of different insecticidal dusts applied to the soil against the young larvae of *L. consanguinea*.

Treatment	One application only (end of May)			Two applications (end of May and in July)		
	Insecticide/ acre (lb.)	Larvae/ acre in Sept.	Reduction (%)	Insecticide/ acre (lb.)	Larvae/ acre in Sept.	Reduction (%)
BHC	10	800	71.4	20	400	85.7
BHC	5	1400	50.0	10	1600	42.9
Aldrin	10	2000	28.6	20	1000	64.3
Aldrin	5	2400	14.3	10	1800	35.7
Chlordane	10	1200	57.1	20	1000	64.3
Chlordane	5	2600	7.1	10	1600	42.9
DDT	10	1400	50.0	20	2000	28.6
DDT	5	1400	50.0	10	1000	64.3
Control	—	2800	—	—	2800	—
Least significant difference ($P=0.05$)					643	

The statistical analysis of the data was done by the transformation $\sqrt{x+0.5}$.

BHC dust at 10 lb. per acre gave the best results for a single application. Two applications of this insecticide at that dosage proved more effective. The cost of the BHC was also the lowest.

(ii) *Application of insecticides in sprays.*—For these trials, suspensions of DDT or BHC or heptachlor in an emulsion spray were applied to the soil with a watering can at the time of the rush of adult emergence at the end of May; the top layer of the soil was then either worked by means of (a) a spade or (b) a cultivator or (c) left unworked. The larval population was recorded in September.

TABLE III.

Efficacy of DDT, BHC and heptachlor applied in sprays to the soil against larvae of *L. consanguinea*.

Series	Treatment	Actual quantity of insecticide/acre (lb.)	Method of working into soil	Larvae/acre in September	Reduction (%)
a	DDT suspension	10	Spade	908	34.20
	BHC suspension	10	"	996	27.83
	Heptachlor emulsion ..	10	"	996	27.83
	Control	—	"	1380	—
b	DDT suspension	10	Cultivator	1326	27.33
	BHC suspension	10	"	1652	10.21
	Heptachlor emulsion ..	10	"	1471	20.05
	Control	—	"	1840	—
c	DDT suspension	10	No mixing	2246	—3.65
	BHC suspension	10	"	1786	17.58
	Heptachlor emulsion ..	10	"	1686	22.20
	Control	—	"	2167	—
	Least significant difference ($P = 0.05$)			397	

The efficacy of these insecticides when applied as liquid to the soil surface was rather low (Table III). The method of working the insecticides into the soil did not make much difference. DDT gave better results than the other two insecticides, but when it was applied to the surface without working into the soil, it failed to bring about any reduction in the larval population.

(c) *Cultural control.*

Under conditions obtaining in Tanganyika, Jepson (1956) has suggested (as a cultural method of control against the chafer beetles infesting sugarcane) "reduction in number of ratoons and then cleaning the ground with a quick growing crop such as white mustard or beans, ploughed in as a green manure," before planting a fresh sugarcane crop. At Dalmianagar, attempts to grow paddy before sugarcane in sandy soils did not prove successful since these soils did not retain enough moisture for the growth of paddy. Growing a winter crop like wheat or barley and keeping the field fallow from June to October, however, considerably reduced the larval population. Leaving heavily infested areas fallow for two years consecutively also helped in reducing the larval population in these fields.

(d) *Varietal resistance.*

Observations were made on a number of varieties, viz., Co.617, Co.527, Co.S.416, Co.935, Co.449, B.O.3, B.O.32, B.O.33, B.O.34, B.O.35 and B.O.38, grown at the Bank Farm, Dalmianagar. It was found that Co.935 and B.O.3 could stand the attack of the larvae better than the other varieties. No variety was, however, found completely immune to attack of the pest.

Parasites and predators.

Parasites.

In September 1958, a Scoliid, *Scolia aureipennis* Lep.*, (fig. 3) was observed to parasitise the larvae in the soil. The rate of parasitisation recorded was only about 5 per cent. The adults of this species are black with a metallic tinge over the wings. They measure 20 mm. in length and 35 mm. in wing expanse. The females were seen in the evening hours burrowing in the moist sandy soil near the infested cane clumps. They made a circular hole, throwing out the soil in small pellets. The beetle larvae are at a depth of about 15 to 30 cm. in the soil at that stage. A larva is first paralysed by the female parasite and then an egg is deposited



Fig. 3.—*Scolia aureipennis*, parasite of larvae of *L. consanguinea*: male, $\times 2.4$.

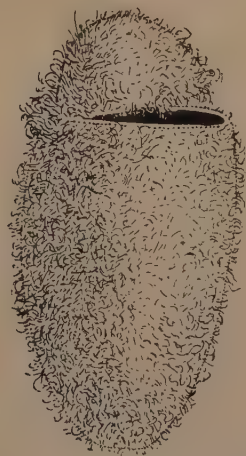


Fig. 4.—Cocoon of *S. aureipennis* after emergence of adult, $\times 2.8$.

on its body. The parasite larva on hatching feeds externally on its host and, when fully grown, pupates inside a reddish-brown cocoon that it spins. The cocoon (fig. 4) is enveloped in a mass of loose brown threads. It measures 20 to 22 mm. in length and 8 to 10 mm. in breadth. The remains of the beetle larva are sometimes found attached to the parasite cocoon. The adult parasite emerges by cutting cleanly a circular 'cap' at the anterior end of the cocoon. Attempts made to rear the parasite in the laboratory did not succeed.

A disease caused by a fungus, first noticed by G. N. Rao (unpublished), was observed to occur in the adults collected from various food-plants. The affected adults became inactive and died in 4 to 6 days' time, and their body contents dried up. The fungus appeared as a white cushiony outgrowth in the body crevices and produced dark green spores. Healthy beetles when inoculated produced symptoms of the disease and died in 4 to 6 days' time. This fungus was identified as *Metarrhizium anisopliae* by the Laboratory of Insect Pathology, University of California (Rao & Vijayalakshmi, 1959).

* A second species, *Campsomeris collaris* (F.), was also represented in material submitted to the Commonwealth Institute of Entomology.

Predators.

The common Indian toad (*Bufo melanostictus*) was observed to prey upon the adult beetles. The toads congregate in large numbers under the trees and bushes and near lights during the flight period of the beetles.

The gecko (*Gecko gecko*) was also observed to prey upon the adults. The geckos took up positions near small bushes and caught the beetles when they came there to feed.

A number of species of birds, particularly the Indian crow (*Corvus splendens*) and the mynah (*Acridotheres tristis*), preyed upon the larvae when the latter were exposed during tillage operations. But since such operations are carried out only in fallow fields, predation by these birds was of little advantage in so far as sugarcane was concerned.

Summary.

The larvae of the Melolonthid beetle, *Lachnosterna consanguinea* (Blanch.), have been found to be very destructive to sugarcane in the Dalmianagar area in Bihar, India, since 1956 when they were first recorded there. The pest has since been found to occur in serious numbers in certain adjoining areas and also in Bihta in Patna district. Damage is done by the larvae by feeding on cane roots. Heavily infested cane clumps dry out completely and in severe infestations as much as 80 per cent. of the crop is lost. The infestation occurs only on light sandy soils; crops on clay soils are not affected. The February-planted crop suffers more seriously than that planted in October. The adults have not so far been observed doing any damage to sugarcane.

The emergence of adults starts in the last week of April, but a rush of emergence occurs only after the first shower of rain. The adults are nocturnal in habit and feed on leaves of certain bushes and trees during the night. They hide themselves in loose moist soil in day-time. Mating takes place after dusk during the flight season, and eggs are laid in the soil at a depth of 5 to 10 cm. The incubation period varies from 8 to 10 days. The young larvae move to cane roots after some initial feeding on grass roots. They are fully grown in 8 to 10 weeks' time when they move deeper into the soil and pupate in earthen cells at a depth of 0.3 to 1.5 m. The pupal period varies from 12 to 16 days and the pest overwinters in the adult stage. There is only one brood a year.

The adults are active at night and are attracted to artificial light and can be destroyed in large numbers with the help of light-traps. Collections from the foliage of trees and shrubs at night is an easier and better method of destruction. Trials with insecticides applied as sprays to the foliage of shrubs in cages to which adults were immediately introduced showed that a suspension containing 0.5 per cent. DDT was effective and was superior to one of BHC.

Replicated field trials carried out for the control of the larvae with various insecticides showed that BHC applied to the soil in a dust at the rate of 22.4 lb. toxicant per acre was the most effective. This treatment, when applied at planting time (February) had an adverse effect on germination; this effect was not observed when the application was made at the end of May. In subsequent replicated experiments, when BHC in a dust was applied in two instalments at the rate of 10 lb. per acre at the end of May at the time of the rush of adult emergence, and in July, respectively, much better results were obtained without any adverse effect on the crop. BHC and other insecticides applied to the soil in sprays at 10 lb. per acre at the end of May were ineffective.

Amongst varieties of cane grown at the Bank Farm, Dalmianagar, Co.935 and B.O.3 showed a greater tolerance of injury by the larvae than the others.

A Scoliid, *Scolia aureipennis* Lep., was observed to parasitise the grubs during September. The rate of parasitisation was only about 5 per cent.

A disease caused by a fungus, *Metarrhizium anisopliae*, was also observed to occur in the adults. Affected beetles died within 4 to 6 days.

The common Indian toad (*Bufo melanostictus*) and the gecko (*Gecko gecko*) were seen preying on the adults in the evening when these were active. A number of birds, particularly the Indian crow (*Corvus splendens*) and mynah (*Acridotheres tristis*), also feed on the larvae when these are exposed during tillage operations.

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OBSERVATIONS ON THE BIOLOGY AND CONTROL OF THE CABBAGE
STEM WEEVIL, *CEUTORHYNCHUS QUADRIDENS* (PANZ.),
ON TROWSE MUSTARD (*BRASSICA JUNCEA*).

By A. L. WINFIELD

National Agricultural Advisory Service, Brooklands Avenue, Cambridge.

The cabbage stem weevil, *Ceutorhynchus quadridens* (Panz.), is one of a complex of pests which attack seed crops of mustard grown for condiment manufacture in East Anglia (Winfield & Gough, 1959; Winfield, 1961a, 1961b). Most of the previous work on the biology of the insect has been done on the continent on other brassica crops (Körting, 1942; Günthart, 1949; Dmoch, 1959), and Wright & Buxton (1957) describe experiments in England on the chemical control of stem weevil on cabbages grown in seed-beds. Large numbers of weevil larvae in leaf petioles and stems cause excessive wilting and loss of mechanical support when cabbage seedlings are planted out, and many of the plants succumb. Mustard is not transplanted and receives no check in growth from sowing until harvest, and the plants are usually very vigorous and growing rapidly when attacked, which may mask the effects of the pest to some extent.

Trowse mustard (*Brassica juncea*) is commonly infested by stem-weevil larvae, but fairly good yields of seed are often obtained from crops in which a high proportion of the stems show apparently severe internal damage. Several mustard growers in the eastern counties complained that stem-weevil damage was one of the causes of reduced seed yields in 1957 and 1958, and it therefore seemed to be important to determine the pest status of stem weevil on mustard, and if possible to devise a suitable chemical control.

Methods.

Most of the observations were made on insecticide trials on Trowse mustard carried out at Benwick, Isle of Ely, and at Trumpington, Cambridge. All the trials were of randomised-block design; in 1958 and 1959, γ BHC and phorate seed dressings were used at high rates at Benwick, and, in 1960, seed dressings and dieldrin sprays were tested on separate trials at Trumpington. The details of these experiments are given later in this paper. From each plot, small numbers of plants were examined for eggs and larvae at intervals during May, June and July; samples were small (5 plants and 10 plants per plot in 1959 and 1960, respectively) because the preliminary trial in 1958 suggested that relatively large differences in infestation between treated and untreated plots might be expected (see also Wright & Buxton, 1957). In the laboratory, each leaf was carefully removed, working upwards from the base of the plant, and the petioles were dissected until one or two uninfested leaves had been found. Experience had shown that above this there were usually no eggs or larvae, and few adult feeding holes. The stems were also dissected, and records made of leaf scars where leaves had fallen, of stem, and occasionally leaf-petiole, diameters, of feeding holes on leaves made by the stem-weevil adults, of egg-laying holes and of numbers of eggs. Counts were also made of stem-weevil larvae (with instar), of cast head capsules and of larval exit holes in the stem walls.

Biology of *C. quadridens* on mustard.

Spring feeding and egg-laying.

Mustard crops are invaded by the overwintered adults as soon as conditions are favourable during April and May (Günthart, 1949; Dmoch, 1959), and on the earlier-sown crops this invasion normally coincides with the start of rapid growth. Dmoch states that the weevils feed for short periods at temperatures above 10°C., but that long flights, and full feeding and sexual activity, take place only above 15°C.

There seem to be two distinct kinds of adult feeding on mustard in spring; general feeding on the leaf lamina, and feeding on the leaf petiole, probably associated with egg-laying.

Feeding areas on the leaf lamina resemble those of *C. pleurostigma* (Marshall) on cabbages (Dunning, 1948), or of *C. contractus* (Marshall), or turnip flea-beetles (*Phyllotreta* spp.), though the holes in the leaves are smaller and more regular in outline than those caused by flea-beetles. Whitish-green spots are made on the broad-leaf laminae and often there is a complete 'shot-holing', but cotyledons are rarely damaged. The heaviest feeding on mustard occurs on the first five broad-leaf laminae, though some feeding was noticed as high as the eighth leaf.

Irregularly-shaped open cavities were seen on the lower leaf petioles immediately before egg-laying began, and were found together with true egg-holes, throughout the egg-laying period, on petioles progressively higher up the plants. The egg-holes are bottle-shaped cavities cut into the leaf petioles, covered by a flap of epidermal tissue beneath which the eggs are inserted, though not all the holes of this kind contained eggs. At first the egg-holes are difficult to find, even with a binocular microscope, but they soon swell slightly and become translucent rounded blisters 1-2 mm. in diameter. There is a distinct round hole at one edge of the blister with a diameter slightly exceeding that of the weevil's rostrum. Frequently the epidermis cracks round the egg-hole as the petiole increases in girth, and the callus tissue produced by the plant in reaction to the weevil's feeding proliferates (Dmoch, 1959). The eggs then protrude from the hole, and Dmoch states that on rape many eggs are squeezed out and perish by exposure.

On mustard, the number of eggs per egg-hole varied from one to nine and averaged two to three; similar numbers in rape plants are reported by the continental workers. The total number of eggs laid by each female may depend to some extent on the host-plant; two females remained alive for the whole of the egg-laying period in 1960 on caged mustard plants in the insectary at Trumpington, and their total progeny were 204 and 195, respectively. A third female died, but 112 eggs and larvae had been found in plants from her cage by 16th May. Speyer (in Dmoch, 1959), Körting (1942) and Dmoch give figures of 140, 281 and 124 eggs, respectively, although the last-named presents evidence to show that under laboratory conditions ten female weevils confined on a single rape plant laid only two to three times as many eggs as did one female on a rape plant. It also seems that the age of the leaf influences the pattern of egg-laying, because the maximum numbers of eggs and larvae on mustard were consistently found a few leaves above the lowest senescent leaf (see also Table II, below). In 1958, there were fewer larvae in the smaller than in the larger plants, presumably because fewer eggs were laid in them. It is not clear how much this was due to differences in the age of the plants and how much to preference of the egg-laying females for larger plants (Table I).

Larval infestation and damage.

On hatching from the eggs, the larvae tunnel in the petioles from the egg-hole, moving at first in any direction but later towards the main stem. The mines are at first barely traceable watery lines in the connective tissue of the petiole, but later they become brown and interconnected, and the inside of the petiole becomes

necrosed. Examinations of larvae and of cast head capsules indicated that only the first and second instars were spent in the leaf petiole; larvae of all three instars were found in the main stems, though second- and third-instar larvae were most numerous. The movement of the larvae from the leaf petioles into the

TABLE I.

Plant size and stem-weevil larval infestation.

Size group	Number of larvae per plant (16.vii.1958)	Number of plants dissected
Stems 3 mm. diameter and under ..	0.22	202
Stems 4-6 mm. diameter inclusive ..	1.68	320
Stems 7 mm. diameter and above ..	4.78	64

Stem diameter measured 5 cm. above soil level.

stems may be inferred from Table II. The proportions of eggs and larvae are shown in fig. 1 (Cl), but the means per plant given in Table II do not agree with those shown in fig. 1, because each of the figures in the main body of Table II is itself a mean, and not all the plants had leaves at all positions.

TABLE II.

Movement of stem-weevil larvae from leaf petioles into stems. Plants sown 4.v.1959; 45 plants examined on each occasion.

Leaf position number	Eggs and larvae per leaf petiole or stem		
	15.vi	24.vi	7.vii
14	—	—	0
13	—	—	0
12	—	0	0.04
11	0	1.20	0.60
10	1.18	1.84	1.05
9	2.56	3.30	1.03
8	3.35	2.22	0.55
7	4.24	2.69	0.78
6	1.02	1.51	0
5	0.60	0.80	X
4	0.27	X	X
3	2.00	X	X
2	X	X	X
1 (lowest leaf)	X	X	X
Stem	2.04	4.71	13.62
Mean per plant	17.26	18.27	17.67
Stems with larval exit holes (max. 45)	Nil	2	19

— No leaf examined at this position.

0 No larvae or eggs; the upper leaves were clean.

X All leaves fallen from this position.

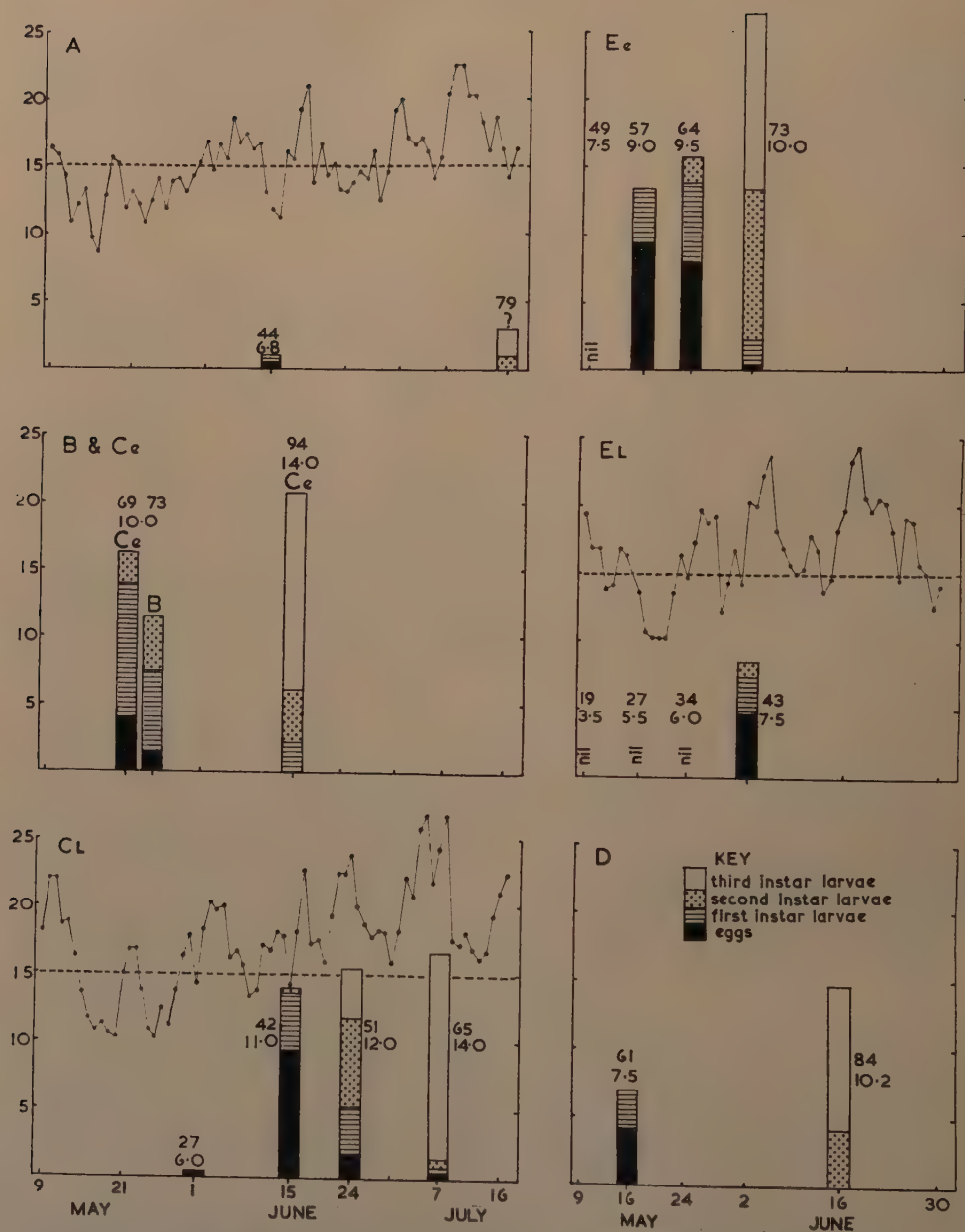


Fig. 1.—Numbers of eggs and larvae of stem weevil in relation to age of mustard plants and mean day-time temperatures, 1958-60. The ordinates scale represents mean day-time temperatures (°C.) for the period 0900-2100 hr. $\left(\frac{\text{Max.} + \text{Min. Tr. for period}}{2} \right)$ and mean numbers of eggs and larvae per plant. A, seed-dressing trial, Benwick 1958 (24 plants per row-ft.); B and C, seed-dressing trials, Benwick 1959 (3 plants per row-ft.); Ce and Cl, counts on early- and late-sown plots, respectively; D, seed-dressing trial, Trumpington 1960 (12 plants per row-ft.); E, spraying trial, Trumpington 1960 (8 plants per row-ft.); Ee and El, counts on early- and late-sown plots, respectively. Mean day-time temperatures (Royal Air Force Station, Wyton, Hunts., approx. 14 miles from either site) plotted as linked dots, one set for each year. The upper of the two figures associated with each histogram represents plant age in days, and the lower, mean number of broad leaves per plant.

As the season progressed there were fewer eggs and larvae at most leaf positions and more in the stems. Some new eggs were laid in the upper leaves, but it seems that egg-laying had almost finished by 15th June because the total number of eggs and larvae per plant did not markedly increase after that date (see also fig. 1 (C)).

On entering the main stems, the larvae mine mainly downwards in the pith from the leaf bases at which they enter, and eventually the mines caused by larvae from several leaf petioles coalesce. In plants severely attacked the whole of the stem from ground level to about 30 cm. high becomes discoloured and hollow, and the epidermis at the stem base silvery and cracked. The damage to the vascular tissue of the stem did not seem to be serious with the larval infestations found under field conditions; when larval numbers were low, the foci of damage often remained localised in the pith round the points of entry at the leaf bases. It was sometimes noticed that the lower leaves of heavily attacked plants seemed to turn yellow and fall prematurely. No larval counts were made in fallen leaves, but according to Dmoch such premature dropping of leaves on rape is responsible for a fairly high larval mortality.

The progress of egg-laying and larval stages recorded at various times on the insecticide trials are shown in fig. 1. The figures for 1958 and 1960 refer only to the records made on untreated control plants; in 1959, the insecticidal seed dressings had only a slight effect and the figures in fig. 1 have been compounded from counts of eggs and larvae on both treated and untreated plants. In 1959, the two trials were about 250 yards apart on the same field at Benwick.

It seems that in spite of there being several days during May when mean day-time temperatures were favourable for egg-laying, that is, above 15°C. (Dmoch, 1959), eggs were not laid in the later-sown plants until they had reached the six- to seven-broad-leaf stage. The earlier-sown plants had reached this stage by early May, and therefore egg-laying began as soon as temperatures were favourable. Adult weevils caged on single mustard plants at Trumpington on 8th April 1960 began feeding on leaf laminae on 11th April, but feeding holes were not found on petioles until late April, and the first eggs on 3rd May. On 4th May, plants from three of the cages were dissected, and there was an average of seven broad leaves, 13.5 egg-holes and 28.0 eggs per plant.

It can also be seen from fig. 1 that larval populations were seldom higher than 26 per plant, and that numbers per plant were generally lower in 1958, when plant population was high. Commercial crops frequently have double the plant population of the 1960 spraying trial.

The larvae leave the stems either by enlarging entry holes in the leaf bases, or by boring new exit holes in the stem wall. It seems that more than one larva uses the same exit hole, because larval counts were always somewhat higher than the number of exit-holes per plant (see also Dmoch, 1959). No critical observations were made on pupae or on the emergence of new adults, but there were numerous weevils of the summer generation in field crops, and on the trials, from about mid-July onwards. Such adults do not lay eggs, but after feeding for a time on wild and cultivated Cruciferae, they seek hibernation quarters in early autumn (Günthart, 1949).

Chemical control and damage assessment.

Seed dressings.

Wright & Buxton (1957) dressed cabbage seed at the rate of 1 oz. of 75 per cent. γ BHC per pound of seed (approximately 4.7 per cent. of the seed weight), and obtained 76 per cent. uninfested plants, compared with 7.6 per cent. on the controls. It was decided to use this method in a preliminary trial in 1958, and, as the results were promising, two further trials were carried out in 1959 on peaty-loam soil at Benwick, and one in 1960 on light, mineral soil at Trumpington. It

TABLE III.

The effect of insecticidal seed dressings on stem-weevil larval infestation and on yields of seed, 1958-60. The insecticides are given as percentages of the weight of seed. Figures in brackets show estimated numbers of eggs and/or larvae in thousands per acre on the date given, which is that on which the maximum number of eggs and/or larvae was recorded. The individual trials and sowing dates are identified by their code letters (see fig. 1, caption).

Trial A (1958)	10% γ BHC	5% γ BHC	Control	Standard error of the treatment means	Residual d.f.
Stem-weevil larvae per plant, 16th July	0.45 (104.5)	1.61 (374)	2.85 (662.1)	± 0.206	6
Trial B (1959)	10% γ BHC	5% phorate	Control		
Stem-weevil eggs and larvae per plant, 25th May	8.6 (790.9)	12.1 (1112.8)	14.3 (1315.1)	± 1.79	10
Yield of seed per plant (g.)	11.71	13.73	12.73	± 0.249	10
Trial C (1959)	Early sown (Ce)			Late sown (Cl)	
Stem-weevil eggs and/or larvae per plant, 15th June	10% γ BHC	5% phorate	Control	5% phorate	Control
	17.1 (1572.6)	25.1 (2308.3)	20.3 (1866.9)	16.7 (—)	11.7 (—)
Yield of seed per plant (g.)	7.96	10.72	8.80	3.95	4.40
Trial D	5% γ BHC	3% phorate	5% Telodrin	Control	
Stem-weevil larvae per plant, 16th June	3.4 (475.7)	17.5 (2458.6)	14.1 (1972.9)	14.7 (2056.8)	± 2.07
Yield of seed per plant (g.)	3.5	2.4	2.7	2.7	± 0.21

was hoped to control the stem weevil without interfering with the two other main pests, blossom beetle, *Meligethes aeneus* (F.), and cabbage seed weevil, *C. assimilis* (Payk.), and in this way assess the effect of stem weevil on the yields of seed.

All the seed-dressing experiments were of randomised-block design and hereafter the letter code, as shown in fig. 1, is used for each trial. In 1958, seed was sown in rows 18 in. apart on 29th April at Benwick (Trial A), the plots being 12 ft. square. High and low rates of γ BHC seed dressing (10 per cent. and 5 per cent. of the seed weight, respectively) were compared with untreated controls, and there were four replications of each treatment. Seed was sown at approximately 8 lb. per acre, or roughly double the commercial rate, giving a population of 24 plants per row-foot. On two occasions, two separate 1-ft. row samples of plants were taken from each plot and were dissected for stem-weevil eggs and larvae.

In 1959, there were two separate experiments about 250 yd. apart on the same field at Benwick (Trials B and C). There were three seed treatments, 10 per cent. γ BHC, 5 per cent. phorate and untreated controls, and on Trial C half the plots were sown on 13th March (Ce) and the remainder on 4th May (Cl). All the plots on Trial B were sown on 13th March. The plots were 9 ft. \times 6 ft. in size and in Trial B there were six replications of the three treatments, and in Trial C three replications of six treatments.

The dressed seed did not run freely in the drill in 1959, and, because the subsequent plant population on the treated plots was lower than that on the controls, all the plots on both trials were thinned by hand to leave three plants per row-foot, or approximately 114 plants per plot. Unfortunately, the late-sown plots of Trial C were thinned on 15th June, and because there were more plants per unit area on the controls, and as stem-weevil egg-laying was almost finished (Table II; fig. 1 (Cl)), a reliable estimate of the number of larvae per acre was not obtained (Table III). The early-sown plots (all Trial B and half of Trial C) were thinned during early May, before egg-laying began, and subsequent egg- and larval-population studies were unaffected. Samples of five plants per plot were dissected for weevil eggs and larvae at the dates shown in fig. 1 (B and C). Small samples were taken because the 1958 trial gave promising results (see Table III) and large differences in infestation were expected between treated and untreated plots.

In 1960, a seed-dressing trial was carried out at Trumpington (Trial D). There were three seed treatments, 5 per cent. γ BHC, 3 per cent. phorate, 5 per cent. Telodrin,* and untreated control. Seed was sown by hand on 25th March on plots 3 ft. square, giving two rows of plants in each plot. The plants were thinned to 12 per row-foot in early April, well before the stem weevils began to lay eggs. Samples of 10 plants were examined from each plot on two occasions for weevil eggs and larvae. The dates of examination are shown in fig. 1 (D).

No seed yields were obtained from the 1958 seed-dressing experiment because livestock from a neighbouring field trampled the plots immediately before harvest. In 1959, yields were assessed by taking 25 plants from an area of one sq. yd. in the centre of each plot. The plants were threshed on a small mobile threshing drum. In 1960, ten plants were taken at random from each plot and threshed by hand. The main results of the three years' trials are given in Table III and include, for each trial, the highest figure for eggs and/or larvae that was recorded.

The seed dressings gave variable results, although in 1958 and 1960 γ BHC reduced the numbers of stem-weevil larvae per plant compared with the controls; in 1960 the plants treated with γ BHC also yielded significantly more seed than the others. On 25th May 1959, significantly fewer eggs and larvae were present in the plants treated with γ BHC compared with the controls of Trial B, but on Trial Ce there were no significant differences between the three treatments by the 15th June. This suggests that γ BHC may have delayed, but did not prevent,

* The name coined by Shell Chemical Co. to denote 1,3,4,5,6,7,8,8-octachloro-3a,4,7,7a-tetrahydro-4,7-methanophthalan, formerly known as WL 1650. The technical material is 97 ± 1 per cent. pure.

egg-laying on the early-sown plants. The results of egg and larval counts from relatively late-sown plants (Trial A and Trial Cl) were also inconsistent. Bardner (1960) has shown that the kind of sticker used, and the formulation of insecticidal seed dressings, may have an effect on the biological activity of the insecticide. The same formulation of the insecticides was used on the mustard in all three years, but the stickers varied, kerosene being used in 1958 and 1960, and a dilute aqueous solution of methyl cellulose in 1959. Apart from any differences that may have been caused by seasonal variations and differences in soil type from site to site, the use of the different sticker in 1959 may have had some effect both on the rate of release of the insecticides and on their toxicity to both plants and insects. It is possible, for instance, that some of the yield differences within trials in 1959 may have been due to some direct action of the insecticides on the plants. MacLagan (1957) demonstrated that seed dressings containing γ BHC or dieldrin could stimulate or depress seedling growth, but the rates of insecticide which he used were lower than those used in the mustard trials.

In 1959, Trial B received a single spray of DDT (3 pints of 25 per cent. emulsion per acre at high volume) against blossom beetles on 27th May; Trial C was unsprayed. The seed dressings had no effect on the damage caused by either blossom beetles or cabbage-seed weevils, but the DDT spray gave a partial control of blossom beetles on Trial B. This is reflected in the higher seed yields of Trial B compared with the early-sown plots of Trial C (Table III), and the greater number of pods set after blossom-beetle damage was finished (Table IV).

TABLE IV.

Damage by *M. aeneus* and *C. assimilis* on the seed-dressing trials, 1959.

Trial				Number of pods per plant after damage by <i>M. aeneus</i> finished	% of pods showing exit holes of larvae of <i>C. assimilis</i>
B	355.7	13.78
Ce	311.9	8.11
Cl	162.7	5.33

Five whole plants from each plot, examined in July (no distinction made between γ BHC, phorate or control plots).

The early-sown plants of Trial C produced roughly twice as many pods as the late-sown plants, which consequently yielded much less seed (Table III). On Trial B, which received the DDT spray, a higher number of larvae of *C. assimilis* emerged from the pods than on Trial Ce; it is possible that the spray killed some of the parasites of the weevil and that therefore more larvae completed their development and emerged from the pods, but it is hoped to discuss this important topic more fully in a future paper. The late-sown plants of Trial C were less heavily infested than the early-sown plants (see also Winfield, 1961a), but the small differences in infestation probably had only a slight effect on the yield differences shown in Table III. Also, the effect of cabbage seed weevil on yield was masked by the relatively large effects of sowing date and blossom-beetle damage.

Sprays.

Wright & Buxton (1957) found that lindane suspensions gave good control of stem weevil when applied to cabbage seedlings in the 2-3 broad-leaf stage, but that dieldrin suspensions were not satisfactory. In 1959, spray-timing trials were

carried out on mustard in East Anglia against blossom beetles and cabbage seed weevils, using dieldrin at the rate of 2 pints 15 per cent. miscible liquid concentrate in high-volume spray (100 gal./acre) to give 0.038 per cent. active ingredient (Winfield, 1961a). A single spray applied during mid-May at the 'green-bud' stage reduced the percentage of stems infested by weevil larvae to 49.2, compared with 71.4 on the controls (averages of 6 sprayed and 12 control plots).

A spraying trial was therefore carried out at Trumpington in 1960 (Trial E). Seed was sown at two dates, 20th March and 21st April (Ee and E1, respectively, in fig. 1), on plots twelve feet square, with a 3-ft. discard between each plot. Dieldrin, at the same concentration and spray volume per acre as in 1959, was applied to half the plots of each sowing date on the 9th, 20th and 24th May. The four treatments were randomised and replicated five times. On four occasions (fig. 1, E and Table V) ten plants per plot were examined for stem-weevil eggs and larvae. On the first examination (9th May), there were no eggs, but numerous feeding holes were found in the petioles, and the first spray was therefore applied. Ten plants per plot were examined after petal fall for blossom-beetle damage, and 100 pods per plot were opened in late July in order to assess larval attack by cabbage seed weevil. Apart from a slight reduction in blossom-beetle damage on the early-sown sprayed plots, there were no significant differences between treatments for these two pests. Yields of seed were obtained from ten plants taken at random from each plot. The plots were not netted until early July, by which time the early-sown plants had been severely damaged by seed-eating birds, mainly sparrows, linnets and other finches. Yields of seed were obtained from plants only slightly damaged, but these plants were probably the least forward, and the yields of the early-sown plots shown in Table V should be viewed with caution. The netting prevented serious bird damage on the late-sown plots.

TABLE V.

Control of stem weevil by dieldrin sprays, Trumpington, 1960 (Trial E).

Treatment	Stem-weevil eggs and larvae per plant (means of 50 plants)			Estimated number of eggs and larvae in thousands per acre 2.vi	Yield of seed per plant (g.)
	17.v	24.v	2.vi		
Early-sown control ..	13.4	15.8	27.3	2546.5	3.29
Early-sown sprayed ..	2.1	3.3	1.4	130.6	3.68
Late-sown control ..	0	0	8.0	746.2	1.13
Late-sown sprayed ..	0	0	0.2	18.7	1.46
Standard error of treatment means	± 1.55	± 0.95	± 2.18	—	± 0.216
Residual d.f.	4	4	12	—	12

The sprays gave a good control of stem weevil, but the results indicate that the first spray (9th May) on the early-sown plots might have given an adequate control without the second and third sprays; very few eggs were laid on the sprayed plots between 9th and 24th May, whilst numbers on the controls rose rapidly. It seems that the adult weevils were controlled and the females prevented from laying eggs. The bird damage ruined the trial for assessing stem-weevil damage, and the only significant yield difference was between early and late sowing (see also Table III, Trial C). Spraying gave an average yield increase of 11.9 per cent. on the early-sown plots, and 28.3 per cent. on the late-sown plots; these increases

seem large, but statistical analysis showed that the differences were not significant because of the wide variations between individual plots. However, although the actual yield losses due to stem weevil were not accurately assessed, the trial did show that a dieldrin spray can give a good control by preventing egg-laying, and that this method of assessment of damage may be useful in future work. It seems that the best time to apply the spray is when adult feeding holes are first found on the petioles.

Discussion.

In the work on mustard described in this paper, detailed observations were not made on the adults and pupae, but were concentrated on eggs and larvae because it was hoped to associate numbers of larvae per plant with seed loss.

It is difficult to assess accurately the damage caused by each of the three main pests of seed crops of mustard, although a preliminary differentiation between that caused by blossom beetles and by cabbage seed weevils has been attempted (Winfield, 1961a). Relative infestations of several different kinds of mustard have also been studied (Winfield, 1961b), but in the experiments reported in the present paper only Trowse mustard was used. Stem weevil reduces the vigour of mustard plants, but does not kill them nor prevent them from setting seed, and the damage is therefore difficult to assess numerically. The main object of the experiments described in this paper was to estimate the effect of stem weevil without interfering with the damage caused by the other two insects. Wright & Buxton (1957) obtained a good control of stem weevil on cabbage seedlings by using γ BHC seed dressings, and this technique was used on mustard during the three years 1958-60. Unfortunately seed dressings were found to be unreliable on mustard, and their insecticidal activity seemed to vary from year to year and from site to site. Some of this variation may have been due to the effects of different seasons, especially whilst the plants were small in April and May, and also to differences between sites in soil type and condition. Furthermore, the methyl-cellulose sticker used to dress the seed in 1959 may have had some effect on the rate of release and the biological activity of the insecticides (Bardner, 1960); in 1958 and 1960, when kerosene was used as the sticker, γ BHC gave a good control of stem weevil, whereas in 1959 the control was poor. Another disadvantage of seed dressings for damage-assessment trials is the possibility that they stimulate, or depress, seedling growth, and it would be difficult to follow these effects through to plant maturity.

Stem weevil was easily controlled by dieldrin sprays during the egg-laying period and there was evidence that a single spraying, when the first adult feeding holes were found in the petioles, would have been sufficient. Even the blanket spraying had only a slight effect on blossom beetles, and no effect on the cabbage seed weevil, which indicates that this technique may be promising for future studies. Further work could include caged plants artificially infested with various numbers of adult weevils, but Dmoch (1959) has shown that under such conditions the number of eggs laid does not necessarily bear a direct relationship to the number of female weevils present in the cage.

The experiments were carried out at sites known to have high stem-weevil populations and the trials covered only a small area, which may have exaggerated the effects of the pest. Stem weevil is common and widespread in East Anglia, but there was no marked evidence, either from the trials or from observations on field crops, that it would be economically worthwhile as a routine to dress seed or spray specifically against stem weevil on Trowse mustard. However, all the trials had some feature which rendered them unreliable for damage-assessment purposes, and future work may show that in some circumstances the control of cabbage stem weevil is of economic value.

Seed dressings containing γ BHC, such as are used by many growers as a routine precaution against flea-beetles (*Phyllotreta* spp.), may occasionally give a

partial control of stem weevil, and, on April-sown crops, the first dieldrin spray against blossom beetles, during May, will probably also give some control (Winfield, 1961a).

Summary.

During 1958-60, replicated insecticide trials were carried out in East Anglia in an attempt to assess seed losses on Trowse mustard (*Brassica juncea*) caused by larval infestations of the cabbage stem weevil, *Ceutorhynchus quadridens* (Panz.). The overwintered adult weevils invade the young growing crops during April and May, and the eggs are laid in the petioles over a period of weeks, depending both on temperature and crop stage, until about mid-June. Egg-laying began on mustard when the plants had produced six to seven broad leaves, but the actual time at which the first eggs were laid seemed to depend mainly on temperatures during May. In the insecticide trials, infestations averaged up to 26 larvae per plant, and none of the untreated plants entirely escaped attack.

High rates of γ BHC (5 or 10 per cent. of the weight of seed) gave some control of stem-weevil larvae when kerosene was used as the sticker for the insecticide; 3 per cent. or 5 per cent. phorate and 5 per cent. Telodrin (1,3,4,5,6,7,8,8-octachloro-3a,4,7,7a-tetrahydro-4,7-methanophthalan) gave no control. The control by seed dressing was unreliable and is considered unsuitable for assessing yield losses caused by stem weevil.

A single emulsion spray of dieldrin applied during May when the first adult feeding holes were found in the petioles, gave a good control of stem weevil and prevented egg-laying, and this technique seems promising for future work on damage assessment. It is thought, however, that sprays specifically directed against stem weevil would seldom be justified economically on commercial crops of Trowse mustard, but on April-sown crops the first dieldrin spray against blossom beetle, *Meligethes aeneus* (F.), may give some protection against stem weevil.

The damage-assessment experiments gave no reliable indication of the effects of the stem weevil on yields of seed, in spite of their being sited in localities known to have a high weevil population. It was clear, however, that sowing date greatly influenced yields of seed; plants sown in March outyielded those sown in April or early May.

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STUDIES OF THE SAMPLING OF *GLOSSINA PALLIDIPES* AUST.

III.—THE HUNGER STAGES OF MALE FLIES CAUGHT ON CATTLE AND IN MORRIS TRAPS.

By I. M. SMITH* and B. D. RENNISON†

*East African Trypanosomiasis Research Organization,
Tororo, Uganda.*

The general composition of samples of *Glossina pallidipes* Aust. taken by traps and on bait animals has been described (Harris, 1932; Swynnerton, 1936; Jack, 1941; Vanderplank, 1944) but little detailed information on the hunger stages (Jackson, 1933) of male flies caught in these ways is available. Knowledge of the nutritional state of flies so sampled is presumably essential for establishing the mode of operation of the attractant used and in evaluating possible biases to which samples might be subject.

Evidence on the nutritional state of tsetse taken in traps and on bait animals is conflicting. Lamborn (1916) and Vanderplank (1947), the latter using the hunger-stage technique, believed that males concentrated round hosts not only to feed but also to find mates. Jack (1941), using measurement of the gross fat content to determine the degree of repletion, found that the weight of fat in trapped males of *G. pallidipes* was lower than in those captured on a bait animal, whereas in trapped females it was higher. He decided from extensive work that both sexes of *G. pallidipes* came to a bait animal only when hungry. A similar conclusion in respect of *G. swynnertoni* Aust. was reached by Bursell (1961) from analysis of fat contents and residual blood-meals (RBM) of flies captured while probing on a cow.

Earlier papers in the present series (Smith & Rennison, 1961*a, b*) have shown that the numbers of *G. pallidipes* taken and the pattern of catching them differed in Morris traps and on cattle. Cattle were conjectured to attract flies in search of food and traps to attract flies in search of shade or resting sites. Were this so, proportional differences in the hunger-stage categories would be apparent in results with the two types of attractant, provided there were no bias in the assessment of the degree of repletion. Data accumulated in the work mentioned offered an opportunity of examining these two points.

Materials and methods.*Site and design of experiments.*

The experimental area at Lugala, in south-eastern Uganda, the attractants used (Morris traps and oxen), the Latin and graeco-Latin designs of the two experiments, the times they were carried out, the methods of catching and the personnel employed have already been described (Smith & Rennison, 1961*a*).

Hunger stages.

The hunger stage of each non-teneral male was recorded as soon as the fly was caught (if on cattle) or not more than 1½ hours afterwards (if trapped), and followed the classification of Jackson (1933), except that flies, from whatever source, containing fresh blood were noted as 'stage 0'. A fly thus described was regarded as having been in stage IV if caught on an ox, and as in stage I if caught in a trap. All flies taken were killed.

* Now at Trinity College, Dublin.

† Now with Department of Agriculture, Kampala, Uganda.

TABLE I.

Summarised data for non-teneral males of *G. pallidipes* caught on different attractants and by different catchers, Lugala, Uganda. Results expressed as percentages of hungry flies, and values of L_w for hungry (stages ~~III+IV~~ ^{III+IV}) and non-hungry (stages ~~III+IV~~ ^{I+II}) flies.

Expt.	Measurement	Attractant									Pairs of catchers (Expt. 1) or individual catchers (Expt. 2)									
		A	B	C	D	E	F	G	H	I	1	2	3	4	5	6	7	8	9	
1	% hungry	63 67	85 70	84 80	89 71	67 72	55 83	71 73	68 98	— —	89 98	70 69	64 71	— —	— —	— —	— —	— —	— —	
	L _w (hungry)	1.2 1.3	1.9 2.1	1.1 1.0	1.2 1.2	1.1 1.0	1.3 1.3	2.1 2.2	2.1 2.2	— —	1.5 1.6	1.5 1.5	1.5 1.5	— —	— —	— —	— —	— —	— —	
	L _w (non-hungry)	0.7 1.0	1.1 1.7	0.4 0.7	0.4 0.7	0.7 0.6	1.2 0.3	1.6 1.7	1.7 0.5	— —	0.4 0.2	1.2 1.2	1.3 1.3	— —	— —	— —	— —	— —	— —	
	% hungry	81	80	87	89	87	70	71	90	40	81	78	96	79	94	75	86	90	90	
	L _w (hungry)	2.0	0.7	1.8	1.6	1.8	0.6	0.5	0.4	0.3	0.9	1.2	1.1	1.2	1.1	1.0	1.0	1.1	1.1	
	L _w (non-hungry)	1.2	0.3	1.0	0.7	0.9	0.3	0.2	0.1	0.4	0.5	0.7	0.2	0.7	0.5	0.7	0.6	0.4	0.4	

The results from the two replicates of Expt. 1 are represented by roman and italicised figures, respectively.

$L_w = \log (M_w + 1) = \frac{\sum \log (n_i + 1)}{N}$ where n_1, n_2, \dots represent the numbers of flies caught in a series of N observations, and M_w represents the Williams' mean (Haddow, 1960, Appendix).

The attractants consisted of Morris traps (used singly or in pairs) or oxen (used singly).

In Expt. 1 the attractants were: A, pair of traps, one with black-painted and the other with natural-coloured (brown) hessian; B, white ox; C, trap with black-painted hessian; D, pair of traps, each with black-painted hessian; E, trap with natural-coloured (brown) hessian; F, pair of traps, each with natural-coloured (brown) hessian; G, red ox; H, black ox.

In Expt. 2, the attractants were: A, red ox; C, red-and-white mottled ox; D, white ox; E, black ox; B, F, G, H, I, single traps with natural-coloured (brown) hessian.

Treatment of data.

To provide a sufficiency of data for analysis, male flies were combined into two groups: 'non-hungry' (stages I & II) and 'hungry' (stages III & IV). The numbers (n) of each of these two groups caught by each attractant each day at each position were used in analyses after transformation to $\log(n+1)$; this device of Williams (1937) was used because, for each attractant, the standard deviation of the catches tended to be proportional to their mean value.

To avoid the presentation of bulky material, the data from the two replicates of Expt. 1 and from Expt. 2 have been deposited as two Tables in the British Museum (Natural History), together with the corresponding analyses of variance from which the probability values given here are taken. Table I in the text summarises the data from the deposited Tables.

Results and discussion.

The findings for each attractant and for the three pairs of catchers in Expt. 1, which comprised two replicates, are summarised in Table I. In the first replicate, highly significantly fewer males were caught on the white ox than on either of the coloured ones ($P<0.001$), but the variation between the oxen as regards the proportion of 'hungry' flies was not significant. In the second replicate, however, fewest males were taken on the black ox ($P<0.01$) and the proportion of 'hungry' flies was greatest on the black ox ($P<0.001$) and least on the white one.

These anomalous results were almost certainly brought about by the catching ability and judgment of the pair of fly-boys (Pair 1, Table I) that caught from the white ox in replicate 1 and from the black in replicate 2. The two men, who even at the beginning of Expt. 1 were categorising males differently from the other two pairs, became more inclined to classify all males caught as 'hungry' as the experiment progressed. During the first four days of replicate 1 they recorded an average of just under 80 per cent. of the males caught as 'hungry' but this value rose to 95 per cent. for the second four days, and throughout replicate 2 about 98 per cent. were noted as 'hungry'. The drift in these figures contrasts sharply with the consistency of the other two pairs of fly-boys as regards the proportions of 'hungry' and 'non-hungry' males recorded during both replicates, and thus offers some confirmation of the existence of a 'fly-boy effect' bias previously suspected (Smith & Rennison, 1961a, p. 171).

Additional evidence that Pair 1 was at variance with the other pairs in their classification is provided by the trap catches. In the first replicate, Pair 1 tended the single and paired black traps (C & D) on four of the eight days, the single brown trap (E) and the mixed pair (A) on three days, and the pair of brown traps (F) not at all. In the analysis, black traps showed a significantly higher mean proportion of 'hungry' flies ($P<0.01$) than did brown traps. During the second replicate, Pair 1 emptied, among others, the single brown trap (E) on two days and the pair of brown traps (F) on five days. Significantly more 'hungry' flies were recorded in the brown pair than in the single brown trap ($P<0.05$). No useful conclusion on the hunger stage of male flies taken by different attractants could therefore be drawn from the first experiment. The numbers caught in this experiment were insufficient to permit a more detailed examination of the four hunger-stage categories considered separately, so that the exact point at which the fly-boy pairs diverged in their assignments to stages cannot be confidently given.

Experiment 2 was designed to control the possible existence of significant variations between fly-boys in catching ability and in assessment of hunger stage and, by taking these possible biases into account, to re-investigate the main problem. In this experiment (Table I, Expt. 2), significantly fewer males were caught on the white ox than on any of the coloured oxen ($P<0.001$). There was no significant variation between traps as regards numbers of males taken, but traps were decidedly less efficient than cattle as attractants ($P<0.001$). The proportion

of 'hungry' flies in the catch varied little between the different animals used in the experiment. Traps, on the other hand, gave very variable proportions. Two traps (F and G) took similar proportions of 'hungry' flies (about 70 per cent.), but another two (H and I) differed greatly from them ($P < 0.01$) and more so from each other ($P < 0.001$), about 90 per cent. of the catch in trap H consisting of 'hungry' flies, compared with 40 per cent. 'hungry' flies in trap I. These differences between traps did not appear to result from any abnormal variation in daily catches as regards numbers or degree of hunger; the proportions of the two hunger-stage groups in each trap were reasonably constant from day to day and from site to site. Flies were recorded daily at $1\frac{1}{2}$ -hour intervals during the catching time (0800 to 1830 hr.), and the frequency of zero values per $1\frac{1}{2}$ -hr. period precluded the satisfactory examination of the data on this basis. Any effects on the trap catches referable to conditions *within* particular days are, therefore, indiscernible. Since the experimental design generally allowed for any effect of fly-boy bias in hunger-stage assessment, the variation between traps is probably real; although such variation cannot be explained by any obvious peculiarities in the traps, some factor must have been operative to make one more and another less attractive to the 'hungry' fly. Consequently, no generalisation is possible about the mechanism by which non-teneral males of *G. pallidipes* are attracted to Morris traps and induced to enter them.

The fly-boys did not vary significantly in the number of males they caught, but they did so in judging hunger stages. As in Expt. 1; the numbers taken were inadequate to permit analysis either per $1\frac{1}{2}$ -hr. period or of the four categories considered separately, and consequently the point at which the assessors diverged cannot be detailed. Catchers in Expt. 2 understood clearly the criteria for each stage and each was 'examined' in his assessment of the degree of repletion of samples of males of *G. pallidipes* before the experiment commenced. Nevertheless, during the experiment the values recorded by different individuals for the percentage of 'hungry' flies among the total caught varied from about 75 to 96. These variations were not likely to have been due to chance ($P < 0.01$). In both experiments the individuals recording these extreme values were men whose experience and length of service equalled those of the men from whom they differed. Thus, in our experience, hunger staging tends to become subjective in the field, so that experimental designs that will permit the isolation of variation arising from differences between individual recorders must be regarded as essential in much field work with tsetse if erroneous conclusions are to be avoided, unless subjectivity from this source can be otherwise overcome. Jackson (1937) found an error of 15 per cent. between 'experienced workers' in classifying hunger stages in the same batch of males of *G. morsitans* Westw. The greater part of this discrepancy was attributable to borderline cases, which, it was felt, would cancel themselves out in a long series of examinations, to leave a 'net error' of 1-6 per cent. This assertion seems to us not necessarily to follow. Part of the problem lies in the difficulty of ensuring that individuals do not categorise differently, and part in the consistency of an individual from time to time. Moreover, an average error of, say, 3 per cent. may have consequences in a sample obtained by one method which it would not have in another sample procured by a different method.

Considering only the data from Expt. 2, our results show that rather less than one male in six taken on the oxen (which, as already mentioned, yielded fairly consistent results) was 'non-hungry' and are thus hardly in agreement with the concept of Lamborn and Vanderplank (*q.v.*) that males concentrate round hosts not only when seeking food but also to find females. Our samples contained only those flies that actually alighted on the oxen, and there could have been many others in the immediate vicinity that went unrecorded, so that the beliefs of Lamborn and Vanderplank are not conclusively invalidated. Our findings with oxen are close to, but not in full accord with, the views of Jack (1941) and Bursell

(1961), who both considered that tsetse came to bait animals only to feed. Both workers based their interpretations on the measurement of fat content. Buxton (1955) has pointed out, however, that the technique, which he himself initiated, of estimating fat to measure the nutritional state, is open to criticism. Bursell (1961) also, however, found that a number of the flies with low fat reserves taken while probing on a cow contained relatively undigested stomach contents (RBM 12%), which, by the hunger-stage technique, would have placed them in a 'non-hungry' category.

As far as catches from oxen (which probably contain an element referable to the presence of the catchers) are concerned, the apparent conflict of evidence may possibly be resolved when it is considered that three samples can be collected. Depending on the technique of catching, the acuity of the catcher and the position of the animal, samples could contain those males that alight only, those that alight and probe, and those that appear in the immediate vicinity of the animal. The separation with certainty of the two former groups in the field must be doubtful, for oxen at any rate are not usually indifferent to the presence of *G. pallidipes* on the places on the body to which this species is mainly attracted. This fact may account for the differences with oxen described between pairs of catchers in the first experiment. Approximately equal numbers of 'hungry' flies were recorded by each pair, but very much lower numbers of 'non-hungry' by Pair 1. If Pair 1 did not usually react quickly enough to the transient presence on the animal of males not intending to probe one might expect to obtain such figures.

There is no evidence to indicate that males of *G. pallidipes* were necessarily attracted to the traps (or individuals among them) used by Jack (1941) for the same reasons as they are to Morris traps. Even Morris traps, as shown, do not apparently catch similar subsections of the male population. In general terms, however, roughly one male in three taken in our traps was 'non-hungry', as judged by the hunger-stage method. As well as contrasting strongly in this respect with the concurrent ox catches, this finding disagrees sharply with that of Jack (1941), who suggested that trapped males were starving flies. Moreover, about one per cent. of the total trapped males in Expt. 2 were noted as 'stage 0' and in other work (unpublished) we have recorded about 7.5 per cent. 'stage 0' daily among trapped males when large herds (80 to 120 cattle), from which no catches were being made, were present in the experimental area. Jack (1941) does not mention this type of trapped fly, but these latter observations also contradict his data (as well as providing, in the former case, a partial measure of the efficiency of the catchers with the cattle, assuming that all 'stage 0' flies obtained their meals from those two sources). While males in 'stage 0' may indeed have very low fat reserves, they can hardly be attracted to Morris traps as possibly resembling hosts, unless the newly-taken feed was partial. In our trap catches, 'stage 0' was intended to imply fully engorged with fresh blood. Although the majority were apparently in this condition, we did not, unfortunately, define degrees of fullness to the catchers, so that some males might have been partially fed only. This latter possibility alone does not affect the general argument, because 'stage 0' formed a small fraction only of the 'non-hungry' trapped males in Expt. 2. When approximately one-third of a full meal had been taken by *G. swynnertoni* in the laboratory, flies interrupted in the course of feeding would not resume (Bursell, 1960). If this is a phenomenon generally true of tsetse in the field, such partially fed flies may continue to possess comparatively low fat reserves but their behaviour, in the period immediately following a feed, may be more akin to that of the fully fed male. Consideration of the contribution of 'stage 0' males to the elucidation of the mode of attraction of Morris traps must be postponed until a measure of the degree of engorgement is applied to these flies. On balance, when the hunger-stage method is used, it is difficult to accept that Morris traps are attractive to all males of *G. pallidipes* only because they possess attributes which activate the flies

to regard the traps as possible hosts, but, because of the problems of 'stage 0' flies, trap differences (both between types and apparently inherent within a type) and no doubt many other factors (such as season, siting, presence of hosts, etc.), no stress can be laid on the present contrast between Jack's (1941) findings and ours.

When the inability of the catchers to conform in their assessment of the degree of repletion is added to the other major variables present, it is clear that no decisive indication of the mode of attraction of oxen and Morris traps is obtainable from these two experiments. Moreover, the variation between catchers in hunger-stage assessment in the field, the variation between apparently identical Morris traps, and the physiological findings of Bursell (1961), all emphasise the need for a re-appraisal of the hunger-stage technique. Indeed, since measurement of gross ether- or chloroform-soluble contents may possibly also be misleading, a general critique of the methods of assessment of the state of nutrition of tsetse is obviously desirable.

Summary.

When non-teneral male flies of *Glossina pallidipes* Aust. were caught concurrently on oxen and in Morris traps in two experiments in south-eastern Uganda and classified, within 1½ hr. of capture, according to a modification of the hunger-stage categories described by Jackson, individual recorders were found to differ markedly in their assessments. No conclusions regarding the hunger stage of flies taken by different attractants could therefore be drawn from the first experiment, but in the second, which was so designed as to discount possible bias amongst recorders, the proportions of 'hungry' (stage III+IV) flies in catches of males were similar on variously coloured individual oxen, and generally higher than in trap catches, in which the proportions varied widely. As all the traps appeared identical, no generalisation is possible about the mechanism by which non-teneral male flies are attracted to and induced to enter such traps.

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STUDIES OF THE SAMPLING OF *GLOSSINA PALLIDIPES* AUST.

IV.—SOME ASPECTS OF THE USE OF MORRIS TRAPS.

By B. D. RENNISON * and I. M. SMITH †

*East African Trypanosomiasis Research Organization,
Tororo, Uganda.*

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The influence of the frequency of emptying Morris traps on the numbers of flies trapped, the effect and mechanism of escape, if it takes place, and the occasional occurrence of apparently inexplicable differences in the numbers trapped by seemingly identical Morris traps (Rennison, in press) were points considered worth examination in a study of the sampling of *Glossina pallidipes* Aust. Other aspects of this investigation have been presented in previous papers in this series (Smith & Rennison, 1961*a*, *b*, *c*). •

Morris & Morris (1949) standardised trap catches of tsetse to 150 trap-days per month; no indication of the frequency with which traps were emptied was given. Morris (1960) showed catches of examples of *G. pallidipes* summed for various periods of time or as weekly means, based on mainly daily trap emptyings. Morris (personal communication, 1957) expected that catches of individuals of *G. pallidipes* in traps emptied several times a day would be greater than in traps emptied only once daily. The effect would arise partly because frequent removal of flies would prevent some from escaping or being taken by predators, and partly because the catches would be increased by the addition of flies drawn to the trap by the person visiting it. Morris (1950) attributed a 40 per cent. increase in efficiency of traps covered with DDT-impregnated hessian to the fact that trapped individuals of *G. tachinoides* Westw. and *G. palpalis palpalis* (R.-D.) were killed by the insecticide before they had time to escape.

This paper describes experiments made in an examination of some of these points.

Materials and methods.*Experimental area.*

The work was carried out at Lugala, south-eastern Uganda, in an area of secondary dry forest and grassland (Smith & Rennison, 1961*a*).

Traps.

For each experiment, the traps used were taken at random from a group of 15 constructed following the slight modification given by Morris (1960). The natural-coloured hessian of the body and sleeve was renewed for each experiment or replicate, or every eighth day in the longer experiments. Unless otherwise desired for experimental purposes, sleeves were closed by several twists of stout rubber bands. All the flies trapped were killed and stored in tubes for check counts and to ensure their removal from the immediate vicinity of the trap and the experimental area.

* Now with Department of Agriculture, Kampala, Uganda.

† Now at Trinity College, Dublin.

Sites.

Locations were taken at random from positions 50 yards apart along the sinuous edge of thicket that lies immediately adjacent to and south-west of the area used previously (Smith & Rennison, 1961a). Traps were placed with their long axes parallel to and one yard from the thicket edge. In such situations they were in open grassland.

Personnel.

In the first, second and fourth experiments, traps were tended by a Field Survey Assistant or by a senior fly-boy and inspected on occasion by the authors, and, in the third, entirely by the authors. In work of the type to be described, mistakes in trap movements (or modifications) vitiate the experiment; in fact, one experiment, not considered in this paper, had to be abandoned because of such an error. That traps had been correctly interchanged and modified (if required) was checked at frequent visits to the experimental area, one or other author always being present in the general vicinity at relevant times.

Plans of experiments.

The investigations were based on randomised Latin or graeco-Latin squares involving consecutive time periods (usually days), sites and traps emptied after various time-intervals and, on occasion, modified in structure to test certain effects.

Treatment of data.

The general arrangement of the experiments and the bulk data, with their appropriate analyses, have been deposited in the British Museum (Natural History). Text tables are summaries of these. Probability values given in the text for each experiment are taken from the total analyses of variance, which were made in accordance with a previous finding (Smith & Rennison, 1961a, c) that the numbers (n) of *G. pallidipes* caught in Morris traps required transformation to $\log(n+1)$, a device originally used by Williams (1937) in considering catches of nocturnal insects taken in light-traps. Except in the last experiment, which contained accumulated totals of eight days, the numbers (transformed) caught were not big enough to allow analysis with the sexes or the numbers of living or dead flies enumerated separately.

TABLE I.

Values of L_w † for adults of *G. pallidipes* caught at Lugala, Uganda, in Morris traps emptied at different frequencies and set out on a Latin square, 8-12.viii.57.

Emptying frequency					Significance	
A	B	C	D	E		
0.06	1.22	1.29	1.30	0.95	***	<div style="display: inline-block; vertical-align: middle;"> $\left\{ \begin{array}{ll} \text{A v. BCDE} & *** \\ \text{E v. BCD} & * \\ \text{B v. C} & \text{n.s.} \\ \text{C v. D} & \text{n.s.} \end{array} \right.$ </div>

† $L_w = \log(M_w + 1) = \frac{\sum \log(n+1)}{N}$; $n = n_1, n_2, \dots$ = no. flies caught in a series of N observations. M_w = Williams' mean (Haddow, 1960, Appendix).
 A = trap catching from 1830 to 0630 hr., emptied at 0630 hr. daily.
 B, C, D, E = traps catching from 0630 to 1830 hr., emptied every 1½, 3, 6, 12 hr., respectively.
 n.s. = $P > 0.05$. * = $0.01 < P < 0.05$. ** = $0.001 < P < 0.01$.
 *** = $P < 0.001$.

Results.

Experiment 1.

The first experiment was to investigate whether the numbers of flies caught by Morris traps were materially affected by the frequency with which they were emptied during daylight and whether such a trap continued to catch individuals of *G. pallidipes* during the night. This latter feature had not, apparently, been tested. Five traps were used, four catching from 0630 to 1830 hours (East African Standard Time) daily for five days and emptied at either 1½-, 3-, 6- or 12-hour (hr.) intervals. The fifth was set to catch overnight from 1830 to 0630 hr. for five nights.

The results, appropriately transformed, are given in Table I.* The numbers taken varied significantly ($P < 0.001$) with emptying frequencies. As the breakdown of the mean square for the latter showed, very much the greater part of the variation was due to the low figure for the trap catching overnight, only one fly (a female) being taken in five nights. (In another experiment, the results of which are not presented here, no flies were taken by a Morris trap set to catch during the night for several nights.) Of the other traps, the one left, unvisited, to catch for 12 hr. during daylight took fewer flies than the other three. This difference, which accounted for most of the remaining variation between emptying frequencies, was significant ($P < 0.05$).

Three explanations of these findings are possible:—

(i) The result may have been an example of the occasional and apparently inherent difference between individual Morris traps in their ability to attract examples of *G. pallidipes* (Rennison, *in press*).

(ii) With cage-entrance gaps of uniform width ($\frac{1}{4}$ in.) and the free ends of the wire strands turned up to prevent flies walking across the gap, flies may have escaped from all traps in similar numbers but the effect may only have become apparent and significant in the trap left unemptied for 12 hr., until 1830 hr. when the decreasing rate of capture in the evening (Smith & Rennison, 1961b) would no longer have counter-balanced escapes. If escape occurs and is a random event with a constant probability per time unit, the numbers trapped should have decreased progressively with decreasing frequency of emptying; a similar but converse argument applies to the contention that a man may increase the numbers caught in frequently emptied traps by drawing flies to them. Since the numbers trapped did not vary directly with frequency of emptying, the first escape hypothesis seems more probable. Alternatively, escapes, if they occur, may be systematic, as may be inferred from the suggestion (Morris, 1950) that most escapes of individuals of *G. tachinoides* and *G. p. palpalis* took place in the late evening or early morning, when trapped flies were no longer phototropically drawn to the top of the cage; Morris (1960) has also stated that examples of *G. pallidipes* normally escaped in the evening.

(iii) Predation of trapped flies may occur in the late evening, during the night or in the early morning.

Experiment 2.

To investigate possibilities (ii) and (iii) above, four traps were set to catch from 0630 to 1830 hr. and one from 0630 to 0630 hr. daily. Flies were removed every 1½, 3, 6 or 12 hr. from the traps operating only during daylight; the fifth trap was emptied at the end of every 24-hr. period. If flies do escape through the entrance

* In this, as in the subsequent experiments described, variations in the numbers caught between periods of time (usually days) and sites are of little interest as far as the points under investigation are concerned. Removal of variations from these sources merely serves to increase the precision with which the effects of other contributing sources of variation are estimated. It should be remembered, however, that in the nature of these experiments some confounding of all effects is to be expected.

slit of the cage, it is reasonable to suppose that an increase of the gap width would permit a greater number of escapes. Gap widths were, therefore, varied from $\frac{1}{4}$ to $1\frac{1}{2}$ inches (in.) and the free ends of the wire strands were not turned sharply up. Morris & Morris (1949) stated that the entrance slit in the cage should be just wide enough to admit flies and not wide enough to allow them to escape but gave no measurement for its width. Morris (1960), although describing the adjustment of the slit to the right width as of the greatest importance to efficiency in catching, likewise did not say what the width should be. It was, however, usually fixed at between $\frac{1}{4}$ and $\frac{1}{2}$ in. (Morris, personal communication, 1957).

TABLE II.

Values of L_{wt} for adults of *G. pallidipes* caught at Lugala, Uganda, in Morris traps emptied at different frequencies and having entrance gaps of different widths, set out on graeco-Latin squares, 23-27.x.57 (replicate 1) and 20-24.xi.57 (replicate 2).

Replicate	Emptying frequency					Significance†
	A	B	C	D	E	
1	1.04	1.26	0.77	1.12	1.10	n.s.
2	0.59	0.75	0.89	0.61	0.91	n.s.
Replicate	Size of gap					Significance†
	1	2	3	4	5	
1	0.87	1.04	1.19	1.15	1.04	n.s.
2	0.86	0.81	0.54	0.92	0.84	n.s.

† See Table I.

A, B, C, D=traps catching from 0630-1830 hr. each day and emptied every $1\frac{1}{2}$, 3, 6 and 12 hr., respectively.

E=trap catching from 0630-0630 hr. and emptied at the end of each 24-hr. period.

1, 2, 3, 4, 5=entrance gaps of $\frac{1}{4}$, $\frac{1}{2}$, $\frac{3}{4}$, 1, $1\frac{1}{2}$ in. width, respectively.

In neither of the two replicates of this experiment (Table II) did the numbers caught vary significantly either with emptying frequency or with gap width; nor did the trap left with flies overnight contain smaller catches than the others. Thus, when traps were left unmoved in one position for a period not exceeding 24 hr., flies did not appear to be taken by predators or to escape in the late evening, during the night or early morning and, even when presented with the opportunity of escaping through a comparatively very wide entrance gap, offering a minimum of hindrance to walking flies, they did not appear to do so in numbers large enough to make significant differences between trap catches. In addition, the periodic visits of one man did not produce any increase in the catches.

Experiment 3.

Although trapped flies might not escape through the entrance slit of the cage either during the day or at night, confusing variations between traps might be produced by careless erection, by careless manipulation of the sleeves or as a result of damaged cage gauzes. An experiment was carried out simultaneously in three parts, each designed to examine one of three simulated defects. The first part (a) involved traps with different entrance gaps; the second (b), traps with different-sized, circular openings arranged in the sleeves; and the third (c), traps with various

numbers of $\frac{1}{2}$ -in. square holes cut in the cage gauzes. The results are summarised in Table III, and have been arranged in two ways: A (upper half of Table), mean value for given frequency of emptying, taken over all gap widths (a), sleeve openings (b), and frequencies of holes in gauze (c); B (lower half of Table), mean value for given gap width, sleeve opening or hole frequency, taken over all emptying frequencies.

TABLE III.

Values of L_w † for adults of *G. pallidipes* caught at Lugala, Uganda, in Morris traps emptied at different frequencies and having varying degrees of simulated defects, set out on a graeco-Latin square, 1-4.vii.58.

A.

Part of expt.	Frequency of emptying				Significance†
	A	B	C	D	
a	1.47	1.47	1.55	1.36	n.s.
b	1.11	0.78	1.40	1.32	** $\left\{ \begin{array}{l} A+B \text{ v. } C+D \text{ ***} \\ A \text{ v. } B \text{ **} \\ C \text{ v. } D \text{ n.s.} \end{array} \right.$
c	0.99	0.78	0.63	0.60	n.s.

B.

Part of expt.	Degree of simulated defect				Significance†
	1	2	3	4	
a	1.48	1.51	1.45	1.41	n.s.
b	1.02	1.22	1.23	1.14	n.s.
c	0.91	1.02	0.71	0.36	* $\left\{ \begin{array}{l} 4 \text{ v. rest*} \\ 3 \text{ v. } 1+2 \text{ n.s.} \\ 1 \text{ v. } 2 \text{ n.s.} \end{array} \right.$

† See Table I.

A, B, C, D=traps catching from 0630 to 1830 hr. daily and emptied every $1\frac{1}{2}$, 3, 6 and 12 hr., respectively.

a=varying entrance gaps (1, 2, 3, 4 represent gaps $\frac{1}{4}$, $\frac{1}{2}$, 1 and $1\frac{1}{2}$ in. wide, respectively).

b=varying sleeve openings (1, 2, 3, 4 represent sleeves shut, or open $\frac{1}{2}$, 1 and 2 in. in diameter, respectively).

c=varying frequencies of $\frac{1}{2}$ -in. holes in gauze (1, 2, 3, 4 represent 2, 4, 8 and 16 holes, respectively).

In (a), neither the varying width of the entrance gap nor the frequency of emptying produced significant variation in the numbers caught.

In (b), however, there was a significant variation ($P<0.01$) in the numbers trapped. This difference was associated with frequency of emptying and was independent of the condition of the sleeves. If the difference had resulted solely from an increased escape of flies from the traps emptied least often, losses should have been greatest in the traps less frequently emptied. In fact, the reverse was the case, significantly fewer flies ($P<0.001$) being taken by the traps emptied every $1\frac{1}{2}$ or 3 hr. than by those emptied every 6 or 12 hr. No fresh spoor of animals that might have activated the trapped flies (see below) was observed near the traps

at any time. Since the dimensions of the apertures in the sleeves apparently did not affect the size of the catches significantly, some peculiarity of the traps, making one more and another less attractive to individuals of *G. pallidipes*, may have been confounded in the results.

In (c), catches were not affected significantly by frequency of emptying, but there was a small difference, unlikely to have been due to chance ($P < 0.05$), associated with the number of holes in the cage gauzes. This effect was produced entirely by the low counts, presumably caused by escapes, in the cage with 16 holes. That escapes were not shown up by significantly varying mean square for emptying frequencies can be explained by the observation that, under our conditions, individuals of *G. pallidipes* were mainly inactive in the traps until disturbed by the very close approach of a person. Flies were then seen to escape at each emptying, particularly from cages with 16 and 8 holes, in an apparently haphazard way. Had we been able to close the holes in the gauzes at each emptying it is probable that no significant difference between cages would have occurred. A number of escapes at other times also is suggested by the fact that the numbers trapped fell progressively with decreasing frequency of emptying. It seems reasonable to conclude that even a comparatively small defect in the cage gauze is conducive to escape and that the latter is a random rather than a systematic effect unless human or animal movements occur very close to a defective trap.

The actual numbers trapped in part (c) of this experiment were only about half of those taken in part (b), which itself yielded only approximately half the numbers taken in part (a). Thus, if we are prepared to ignore possible effects of site and individual traps, both of which certainly occur at times, this experiment also suggests that possibly faulty sleeve closure, and, more certainly, defects in the gauze of the cage, permit escapes to occur and are, therefore, factors which might seriously influence the comparative performance of traps, contribute to the differences which arise sporadically between traps, and seriously compromise results.

Experiment 4.

Except when traps were badly damaged, only insignificant losses, if any, were encountered in traps catching for periods of up to 24 hr. Other factors might influence comparative performances were the catching period in one site to be more prolonged. In this experiment, accordingly, traps were left for eight days at each of five sites and were emptied either twice a day, once a day, or every second, fourth or eighth day. Five traps (A-E) were used and were moved in rotation around five sites, each remaining in a given site for eight days and being moved on to the next site at 0630 hr. on the first day of the next catching period, of which there were five in all. The experiment was carried out in two parts, in the first of which the widths of the entrance gaps were varied from $\frac{1}{4}$ to $1\frac{1}{2}$ in.; in the second part, these modifications were dispensed with in favour of the isolation of individual trap differences.

The results of part 1 of this experiment are not presented here in detail. The outcome was unsatisfactory because of the loss of an unknown number of flies, which were taken by ants. Traps emptied more frequently caught greater numbers of flies than did those less frequently emptied, a finding which suggested that losses increased with the passage of time, and, in the absence of a significant variation dependent on gap width, that these losses were more likely to have been a result of predation than escape. The data indicated that ants were not equally voracious at all sites or on all days. Their attacks, therefore, must account in part for site, time and trap variations. This was the first instance we encountered of trapped flies being taken by predators in any work we had conducted. Presumably ants were attracted to the traps initially by the presence of considerable numbers of dead flies (or perhaps because of the relatively greater and increasing numbers of flies) within traps left unmoved for comparatively long periods. Despite frequent

visits, it is uncertain if these attacks were observed on every occasion. Tsetse thoraces were usually left in the traps but on at least one occasion no trace of either trapped flies or ants was found, although the former had been present in numbers estimated at about 50 on the previous evening. This startling finding gives cause to consider the efficiency of sampling by Morris traps. Although he worked under conditions very similar to ours, Morris (1960) made no mention of losses of flies through predation. In most of his investigations, as far as can be judged, traps were left in one position for a week and visited (and presumably emptied) once daily, at a time not stated, on five days of each week. He gave no indication of measures designed to eliminate the possibility of losses of flies by predation. Losses obviously occur in traps left in one position for more than 24 hr. Without measures to protect trapped flies from predation,* we would not agree with Morris (1960) that long-term results with Morris traps are necessarily accurate and reliable.

To prevent the incursions of ants, the traps used in the second part of this experiment were mounted on greased, single, central legs. This device was apparently successful; no incursions of ants were observed, the traps being inspected every 2 hr. during daylight and once during darkness, and no partially eaten flies were found in the traps. The results of the second part of the experiment are given in Table IV, in which the results are summarised in four different ways, according to catching period, individual site, individual trap, and interval at which emptying was done.

The numbers taken during the various catching periods differed somewhat ($P < 0.05$), but for traps, sites and emptying frequencies the numbers caught varied in a way attributable to chance alone. Significantly more flies were alive than dead when taken from the traps ($P < 0.01$). The ratio of dead to living among males was just significantly greater than among females ($P < 0.05$); this finding could be a reflection of the lower fat content of trapped males of *G. pallidipes* compared with females (Jack, 1941). Although there was no difference not attributable to chance in the number of deaths recorded in individual traps, a decrease in the frequency with which they were emptied was accompanied, as might be expected, by an increase in the proportion dead, which rose from about 20 per cent. when they were emptied once or twice a day to approximately 80 per cent. when emptied once in eight days. Assuming that the earliest trapped flies died soonest and that the death-rate was about 20 per cent. per 24 hr., the approximate median length of life remaining to flies from the time they entered a trap was about 48 hr. This figure would vary slightly for the two sexes and, as will be shown, more substantially during different catching periods and at various sites. The fat reserves of males were thus apparently adequate to sustain half of the essentially inactive flies for about two days. Likewise, females, probably all pregnant, had apparently only enough fat to support themselves and their larvae for an approximately equivalent period. The relevance of this observation to the population in general is very doubtful since the ecological conditions and behaviour of trapped samples of *G. pallidipes* are unlikely to be at all similar to those of free flies. The result concurs reasonably well with the finding (Smith & Rennison, 1961c) that about one trapped male in three was 'non-hungry' (stage I+II of Jackson, 1933) and contrasts with the observation (Jack, 1941) that males of *G. pallidipes* caught in Harris traps were apparently starving but females comparatively well nourished.

Decidedly fewer deaths ($P < 0.01$) occurred in the second and fourth catching periods (10-18.iv and 26.iv-4.v), being about 30 per cent. in these as opposed to 50 per cent. in the remainder. Similarly, there was significant variation ($P < 0.05$) in the proportion of deaths at some sites, e.g., 60 per cent. at site 2 as opposed to 34 per cent. at site 4 and between 41 and 43 per cent. at the other sites.

* Examples of some species of small rodents also will destroy every fly captured by a Morris trap, if they gain access to the cage (Smith, unpublished).

Since survival differed unaccountably at different sites, speculation on the varying proportion of deaths in different catching periods is of little value. Although not necessarily of small importance, these differences were of low significance, as might be expected to have arisen in catches at times so close together that climatic fluctuations were unlikely to have been affecting the population rigorously. There may be, therefore, another factor or set of factors in trapping in general whose rôle has not yet become apparent.

Discussion.

It might be thought that the problem of the effect and mechanism of escape would be more easily resolved by putting marked flies into traps and studying the outcome. This method appears to us to have certain defects, of which the greatest are the method of capturing and the source of the flies to be inserted, together with the effects of handling and marking on their behaviour. In any event, we were not concerned in this work to show that escape of individuals of

TABLE IV.

Values of Lw^{\dagger} for adults of *G. pallidipes* caught at Lugala, Uganda, in Morris traps emptied at relatively long intervals, set out on a graeco-Latin square, 1959.

			Catching periods				
			2-10.iv	10-18.iv	18-26.iv	26.iv-4.v	4-12.v
♂♂	Alive	..	1.02	1.14	0.99	0.93	0.84
	Dead	..	1.34	0.71	1.02	0.91	0.92
♀♀	Alive	..	1.78	1.59	1.41	1.47	1.29
	Dead	..	1.76	1.21	1.33	1.17	1.20
			Sites				
			2	4	11	17	22
♂♂	Alive	..	0.89	0.98	1.12	1.04	0.90
	Dead	..	1.16	0.68	1.14	1.12	0.80
♀♀	Alive	..	1.47	1.44	1.67	1.47	1.49
	Dead	..	1.36	1.00	1.59	1.51	1.19
			Traps				
			A	B	C	D	E
♂♂	Alive	..	0.92	0.92	0.87	1.05	1.17
	Dead	..	0.93	0.85	0.97	1.14	0.99
♀♀	Alive	..	1.56	1.46	1.41	1.41	1.69
	Dead	..	1.31	1.35	1.26	1.39	1.34
			Emptying frequency				
			1	2	3	4	5
♂♂	Alive	..	1.25	1.23	1.18	0.89	0.38
	Dead	..	0.65	0.74	1.24	1.19	1.08
♀♀	Alive	..	1.77	1.77	1.46	1.48	1.02
	Dead	..	1.00	0.94	1.50	1.66	1.56

[†] See Table I.

Emptying frequencies 1, 2, 3, 4, 5=trap emptied twice daily (at 0630 and 1830 hr.), and daily or every second, fourth and eighth day, at 0630 hr., respectively.

Analysis of variance.

Source of variance			d.f.	mean square	F
Catching periods	(P)	..	4	0.5438	4.82*
Sites	(S)	..	4	0.4273	3.79
Traps	(T)	..	4	0.0990	0.88
Emptying frequency	(F)	..	4	0.3312	2.94
Error		..	8	0.1127	—
Sexes	(X)	..	1	5.4662	134.63***
Alive or dead	(C)	..	1	0.3576	8.81**
XC		..	1	0.1798	4.43*
XP		..	4	0.0342	< 1.0
CP		..	4	0.1862	4.59**
XCP		..	4	0.0206	< 1.0
XS		..	4	0.0187	< 1.0
CS		..	4	0.1359	3.35*
XCS		..	4	0.0177	} < 1.0
XT		..	4	0.0095	
CT		..	4	0.0390	
XCT		..	4	0.0090	
XF		..	4	0.0220	
CF		..	4	1.5706	38.68***
XCF		..	4	0.0259	< 1.0
Error		..	24	0.0406	—

*, **, ***: see Table I.

G. pallidipes does not take place from Morris traps, but rather to investigate the effect of escape (if it occurs) on the comparative performances of Morris traps.

It seems clear that if flies escape they do so randomly and in approximately equal numbers from all traps treated similarly and that, except when a trap was badly manipulated or its cage seriously damaged, escape alone is unlikely to have a decided effect on comparative performances. It also seems that an individual tending traps does not draw flies to them; the grounds for suggesting that this might occur seem doubtful, for there is no indication that the section of the population attracted to man is also attracted to a trap.

The results generally suggest that the sum of catches in frequently emptied traps will be about equivalent to those in traps less frequently emptied in the same period, always provided that traps are well-maintained and kept free of ants.

The results make it obvious that predation by ants is possibly the most important single factor influencing comparative trap performances, as well as possibly being a part of the day-to-day and site-to-site differences which are frequently detected. We did not observe predation until traps were left unmoved in one site for periods exceeding 24 hr. However, since evidence of ant activity would not always remain to be seen, losses of flies could remain unknown. When the entry of ants was prevented, the numbers of flies taken by traps were similar. In these conditions, therefore, if flies escaped they must have done so in approximately equal numbers from each trap, a supposition which seems unsupported, since the numbers caught did not decrease progressively with decreasing frequency of emptying. This being so, the increased efficiency of traps whose hessian covers had been impregnated with DDT might have been due less to the reduction of escapes of individuals of *G. tachinoides* and *G. p. palpalis* (Morris, 1950) than to increased deaths among intruding predators. If these tsetse usually alight on the trap legs, as do examples of *G. pallidipes*, and if they pass as rapidly from the belly of the trap to the cage, the extent of contact with the insecticide must have been minimal. Intruding ants, however, might well have been exposed to much higher doses of DDT.

Individuals of *G. pallidipes* were attracted to, and some actually fed on, bait

cattle during the night (Chorley & Hopkins, 1942; Moggridge, 1948). The numbers caught were not big, but in the circumstances many flies might have been missed. Although more flies were taken on moonlit nights, there was good evidence of much fly activity even during dark nights. Vanderplank (1941, 1948) also reported rather similar conclusions; Williams (1943), however, caught only one fly on a bait ass between 1900 and 0500 hr. In some circumstances at least, therefore, it seems that bait cattle (and man) are attractive to individuals of *G. pallidipes* at night. By contrast, under the conditions in which we worked, Morris traps almost totally failed to catch examples of this species at night, although they took considerable numbers by day. Since trap differences or losses of flies by escape or predation are unlikely explanations of this failure, it is reasonable to suppose that there are conditions under which traps do not represent the natural host (Morris, 1960). Simultaneous comparisons of the attractiveness of bait animals, man and Morris traps during the night, however, would be of value in settling this point.

Summary.

Experiments carried out in south-eastern Uganda, as part of a series of investigations of factors affecting the catch of *Glossina pallidipes* Aust. by Morris traps, showed that this was not significantly affected by varying the interval between emptying the traps within the range 1½–24 hours or by varying the width of the entrance slit from ¼ to 1½ inches. Such exceptions as occurred could be attributed to unexplained variations in the intrinsic performance of individual traps. Almost no flies were trapped at night (1830–0630 hr.).

Catches in traps with simulated defects (leaving the sleeve open or making holes in the cage gauze) were lower than in perfect traps; the catch was not significantly affected by the diameter of the sleeve opening, but was significantly lower in traps with 16 ½-in. holes in the cage gauze as against those with 2, 4 or 8. Escapes through such holes were observed whilst emptying the traps, when the recorder's approach disturbed the trapped flies, which were otherwise inactive. Frequent visits to the traps to empty them did not materially increase the number of flies effectively trapped, as might have been expected were flies escaping continuously or were the traps to catch flies accompanying the observer.

When the interval between emptying traps exceeded 24 hr., losses of trapped flies occurred, to an extent that is unknown but sometimes substantial, as a result of predation by ants; this is the most likely source of significant variation in catches made by apparently identical traps, and can be overcome by mounting traps on greased, single legs. When thus protected, there was no significant difference in catch between traps cleared at intervals ranging from 12 hr. to eight days. The longer this interval, the greater the proportion of dead amongst captured flies; the median length of life after capture was about two days, this period varying slightly with sex and more substantially with undefined differences between sites and trapping periods.

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FIELD EXPERIMENTS ON THE CONTROL OF WHEAT BULB FLY, *LEPTOHYLEMYIA COARCTATA* (FALL.).

By H. C. GOUGH, A. WOODS,* F. E. MASKELL and M. J. TOWLER †

National Agricultural Advisory Service, Brooklands Avenue, Cambridge.

Although damage by wheat bulb fly, *Leptohylemyia coarctata* (Fall.), can be prevented or reduced by various cultural measures these are not always practicable (Gough, 1957) and chemical methods were therefore investigated. Preliminary experiments suggested that seed dressings would be the simplest and most promising approach as Gough & Cohen (1954) found that spraying to kill adults was unsatisfactory.

Seed dressings containing 20 per cent. γ BHC to prevent wireworm damage were introduced commercially about 1949–50, but observations on fields where this treatment was used indicated that it had little effect on attack by wheat bulb fly, and higher rates were therefore tested at the outset. About the same time, aldrin and dieldrin became available for experimental purposes, and seed dressings containing these materials were used in the first trial; other materials were introduced for comparison or as they became available and, in the later stages of the work, chemicals found promising by Bardner (1958, 1959) were included.

Methods.

Fields on commercial farms were usually selected for experiments on the basis of a fairly high egg count of wheat bulb fly, preferably over one and a half million per acre; these egg counts were based on 20 four-inch cores per field, the samples being examined by a flotation process (Cockbill & others, 1945). All cultivations, including sowing, were carried out by the farmer or his staff with his own equipment. Smaller trials were drilled with a hand machine.

Dry seed dressings only were used and all seed was treated in a small barrel-type dressing machine, usually within 24 hours and invariably within a few days of sowing. Except where otherwise stated, all insecticidal seed dressings also contained a suitable quantity of organo-mercurial fungicide and the seed on all control plots in experiments where the insecticide was combine-drilled was treated with an organo-mercurial fungicide (O.M.). The rate of dressing was either 2 or 3 oz./bushel (equivalent to 2 or 3 g./kg.). Where possible, seed-drills were calibrated beforehand so that the sowing rate of the seed or any material combine-drilled would fall within the chosen range. The rates were checked after the drilling of each treatment by noting the difference between the weight of seed or material put in the drill and the residue.

Counts of attacked and healthy plants and shoots were made on sample areas of two adjacent rows each 1 ft. long on one or more occasions and the number and condition of the larvae in the attacked shoots (or a sample thereof) were recorded. The number of samples taken depended on circumstances but was usually three to five per plot. Usually these samples were brought back to the laboratory for examination and dissection under a low-power binocular microscope though sometimes it was possible to make total-plant and damaged-shoot counts in the field.

* Present address: University of Sydney, N.S.W.

† Present address: Fairview, Gt. Chishill, Royston, Herts.

TABLE I.
Details of trial sites.

Expt. No.	Site	Soil type	Previous crop	Plot size (acre)	Date sown	Variety	Seed rate (bushels/acre)	Main sampling dates	Larvae per acre (controls)	Shoots per plant at main sampling date (controls)
1	Woolley, Hunts.	Clay loam	Fallow	1/10	10.xi.51	not recorded	2	18/19, iii.52	160,000	not recorded
2	Stonea, Isle of Ely	Sandy loam	W. wheat (failed)	1/60	16/17, iii.53	Atle	—	22.iv.53	—	—
3a	Whittlesey, Isle of Ely	Loamy peat	Potatoes	1/25	13.xi.53 6.i.54	Hybrid 46	3½-4	iii.54	1,530,000 670,000	2.35 1.0
3b	Crowland, Lincs.	Light acid peat	Potatoes	1/8	29.x.53 17.xii.53	Cappelle Desprez	3½-4	iii.54	1,870,000 710,000	2.1 1.0
4	Whittlesey, Isle of Ely	Loamy peat	Peas	1/25	7.xii.54	Hybrid 46	3	13.iv.55	1,070,000	1.5
5	Trumpington, Cambs.	Sandy loam	Early potatoes	1/200	16/17, xii.54	Hybrid 46	Low (est. 100 lb./acre)	9.iv.55	250,000 (est.)	2.1
7a	Stonea, Isle of Ely	Sandy loam	Early potatoes	1/40	9.xi.55	Hybrid 46	—	19.iii.56 19.iv.56	960,000	1.3
7b	Warmington, Northants.	Clay loam	Fallow	1/20	17.xi.55	Cappelle Desprez	2½	19.iv.56	800,000 (est.)	2.2
8	Trumpington, Cambs.	Sandy loam	Early potatoes	1/240	6.xii.55	Hybrid 46	Low (est. 1-1½)	28.iii.56 18.iv.56	185,000	1.9
9	Newborough, Soke of Peterborough	Loamy peat	Potatoes	1/33	23.x.59 17.xi.59 17.xii.59	Hybrid 46	2½	4.ii.60 31.iii.60	955,200 656,500 367,150	3.3 4.0 2.7

It was apparent in early experiments that to record only presence or absence of larvae in plants was inadequate because some insecticides were killing them after entry. As in most mortality assessments, the criterion of living and dead was not easy and a moribund category had to be introduced. Later experience showed that dead larvae were often difficult to find and that most larvae classified as moribund subsequently died. The best assessment of treatment effect on larvae was therefore the number of live or apparently normal larvae per unit area.

Because of the large number of plants which were collected from various experiments at about the same time, it was necessary to store them at a low temperature (about 1–3°C.) and the examination could then be spread over several weeks. Observations showed that when information was required on the state of the larvae, the plants should be examined within ten days of collection, and under these circumstances the percentage of moribund or dead larvae in samples from the control plots rarely exceeded 10 per cent.

Most field trials were arranged as randomised blocks with all plots parallel, and consisting of one drill width (rarely two) at least 40 yd. long, ranging in area from 1/60 to 1/8 acre. The plots on hand-drilled trials were about 1/200 acre.

Ideally, a plant-establishment count to determine the effect of treatments on germination should be made after maximum plant emergence, but before the larvae have hatched. In nearly all the years under review, frost or snow during January, February or March prevented this, and the first count could not be made until after the plants had become infested. Except when sowing was so late that seedlings were attacked before they had emerged, the total plant count at this stage should still reveal any direct effects of treatment on germination, as wheat bulb fly rarely kills plants so early. Where appreciable underground attack occurred, the sample drills were dug out, but this tedious process usually underestimated both plants and larvae. The main count of wheat bulb fly was made as soon as possible after maximum primary infestation but before there was much movement from shoot to shoot (secondary attack).

General observations on the crop were made during the growing season and on at least one occasion the plots were allotted marks (scored) for general appearance. Where possible, yields were obtained by harvesting each plot separately with a combine-harvester. The hand-drilled plots were cut by hand and threshed in a special machine. The moisture content of grain samples was determined and the results recorded in terms of 15 per cent. moisture content in cwt./acre on field plots and lb./plot on small plots.

All experiments have been numbered serially and more or less identical experiments repeated on more than one site in the same season were given the same number but differentiated by a letter following the number.

The figures selected for presentation of the results vary according to circumstances but usually include:

- (1) Total numbers of plants per unit sample; this figure shows at an early stage any phytotoxic effects of treatment, and at a later stage the effects on infestation also.
- (2) The percentage of shoots attacked, which is the best single measure of the degree of attack; angular transformations of these percentages were used for analyses of variance.
- (3) The percentage of larvae found which were alive or normal; this shows the effect of the insecticide on those larvae which entered the plant. The total number of live larvae per unit area reflects the combination of (2) and (3) and is the best measure of the total effectiveness of the insecticide.

All these figures will vary according to the time at which they were made and therefore they are not necessarily comparable between trials.

Site details of all trials except 6a–h are given in Table I. This includes the mean number of shoots per plant for the control plots at the time of sampling.

This figure is a function of time of sowing and subsequent growing conditions and obviously influences the percentage of shoots attacked. The number of larvae per acre on the control plots is also given as a measure of the potential attack. Numbers well below 500,000 are likely to have little effect, and numbers above 500,000 an increasingly greater effect on yield.

A summary of the results of each experiment is given in turn, and attention drawn to the main conclusions which determined the next stage of the investigation.

Results.

Experiment 1.

The first trial was a comparison of γ BHC, aldrin and dieldrin as seed dressings at 2 oz./bushel in a 5×3 randomised block. The results are shown in Table II.

TABLE II.

Seed dressing	Plants/sample	Total larvae/ sample	Percentage dead larvae	Percentage germination in laboratory tests
γ BHC, 40%	21.2	3.2	0	87.5
Aldrin, 40%	29.2	4.9	7	97.5
Dieldrin, 20%	23.4	2.9	19	93.5
Dieldrin, 40%	21.6	2.9	8	85.0
Control (O.M.)	26.8	4.3	0	Not done

Although there were no visual differences at the time of sampling, the number of plants in the γ BHC plots and in the dieldrin-treated plots are significantly lower (5 per cent. level) than on the controls and aldrin plots. This agrees with the differences in percentage germination in a laboratory test. There were no significant differences in the percentage of damaged plants or shoots or in the number of larvae, though it should be noted that the attack was light. The occurrence of dead larvae in the plants from aldrin- and dieldrin-treated seed was noted as being unusual.

Experiment 2.

The disappointing results of the previous experiment suggested that seed dressing with these materials was not worth pursuing, though the interval between treatment and time of attack was fairly long. The opportunity to test the effects of a shorter interval came in 1953 when a farmer wished to sow spring wheat after a failure of winter wheat due to wheat bulb fly. As most of the larvae were only

TABLE III.

Treatment	Percentage attacked shoots (22.iv)	Plants/sample (7.v)
γ BHC, 40%	16.2	26.7
Aldrin, 40%	8.3	27.2
Dieldrin, 40%	5.4	29.5
Control (O.M.)	30.5	14.4

about half grown when the winter wheat was ploughed in, it seemed possible that they would move to the spring wheat when it germinated. Thus the circumstances of this experiment were quite different from any others. The same materials and rates were used as in the previous trial except that 20 per cent. dieldrin was excluded. The experiment was arranged as a 4×2 randomised block and was regarded as an observation trial; the results are shown in Table III.

Experiments 3a and 3b.

The results in experiment 2 clearly indicated that under certain circumstances seed dressings might be effective in reducing wheat bulb fly attack and it seemed worth investigating their efficiency at various sowing dates.

Experiments 3a and 3b, respectively 6×4 and 6×3 randomised blocks, were therefore designed to compare the effects of 40 per cent. γ BHC and 40 per cent. dieldrin at two different sowing dates in the same trial. The rate of treatment was increased to 3 oz./bushel as it was thought that the wheat would retain rather more dressing at this rate than the more usual 2 oz. rate.

Although the mid-November sowing in Expt. 3a would not normally be regarded as early, the late autumn in 1953 was mild, and in both trials the early-sown wheat had tillered well, masking the heavy larval attack. By April, on Expt. 3a, the late-sown plots of the γ BHC treatments appeared uniformly good, the control plots uniformly poor and the dieldrin plots intermediate. In Expt. 3b, with a very heavy attack, on the late-sown plots the γ BHC treatments were moderate, the controls a complete failure and two of the dieldrin treatments intermediate, the third replicate being poor. In May, visual differences were still apparent on Expt. 3a, but the late-sown dieldrin plots were much improved relative to the late-sown controls which were by then a complete failure; all the early-sown plots were still uniformly good. On Expt. 3b, all the late-sown plots were poor and the attack had caused considerable thinning of the early-sown plots.

The results are shown in Table IV. The early-sown wheat tillered before larval attack, and each plant had two or three shoots compared with only one shoot on the late-sown plants. Furthermore, as only one larva survives in any one shoot

TABLE IV.

			Early sown			Late sown			S.E. of treatment means
			Control	γ BHC	Dieldrin	Control	γ BHC	Dieldrin	
Shoots/sample	a		119	138	133	33	36	29	± 8.0
	b		113	114	114	32	33	28	± 5.9
Larvae/sample (estimate)	a		41.4	27.6	31.8	18.2	6.4	7.2	± 2.3
	b		50.7	43.2	39.0	19.2	19.6	8.7	—
Attacked shoots (per cent.)	a		43.1	34.1	34.3	79.6	37.3	65.0	—
	b		53.8	55.7	53.8	96.4	77.0	74.9	—
Attacked shoots (Angles)	a		40.8	35.5	35.8	63.9	37.5	53.9	± 2.2
	b		47.3	48.3	47.1	79.2	61.3	61.9	± 4.7
Yield (cwt./acre) ..	a		21.8	19.8	21.4	*	21.5	21.7	± 1.2
	b		23.6	25.0	24.3	*	17.3	7.8	± 2.4

* Complete failure and crop not harvested.

(Gough, 1946) the number of larvae in the late-sown plots is limited by the number of available shoots (Raw, 1960), so that the late-sown plots have fewer larvae; nevertheless the percentage shoot attack is much higher owing to the smaller number of shoots.

On the early-sown plots there were no significant differences between any treatments for percentage shoot attack (angular transformation) or yield, though the number of larvae per unit sample was significantly reduced by both treatments in Expt. 3a. There were, however, marked and significant reductions in percentage attack for both treatments on the late-sown plots in both trials, and also a significant difference between γ BHC and dieldrin in (a). Larval numbers were also significantly reduced by treatments in (a), and in both trials the yield on the control plots was not worth harvesting, whereas the yield of the treated plots did not differ significantly from the early-sown plots in (a); in (b) the yield on the late-sown BHC treatment was much lower than on the early-sown plots and that on the dieldrin treatment was poor.

The different effects of γ BHC and dieldrin have already been recorded (Gough & Woods, 1954). Briefly, it appeared that γ BHC prevented many larvae from entering the plant, but most of those which entered, survived; dieldrin only slightly reduced the number entering but subsequently many of them died. This explains the poor appearance of the dieldrin plots initially and their subsequent improvement. It was apparent that in future trials the larvae would have to be carefully examined within a limited period and their condition recorded.

This experiment established the importance of sowing date as a factor in the effectiveness of seed dressings, but it also suggested that these seed dressings could not always be relied upon to give a fully effective control of wheat bulb fly. Three courses suggested themselves:

- (1) to test other insecticides as seed dressings,
- (2) to increase the concentration or load of insecticide on the seed (see Bardner, 1959),
- (3) to apply greater quantities of insecticide near the seed by combine-drilling.

In 1954, the only other insecticide tested was aldrin and at that time it proved impossible to obtain concentrations of aldrin or dieldrin higher than 50 per cent. In view of the risk of phytotoxicity with γ BHC, we did not think it advisable to increase the concentration above 40 per cent., and because of the risk of taint to subsequent potato crops, we did not use γ BHC as a soil treatment.

Experiment 4.

Experiment 4, a 5×4 randomised block, was designed to compare aldrin and dieldrin as seed dressings with dieldrin combine-drilled at approximately 1 and 4 lb./acre.

There was an initially severe attack apparent on all plots in April, but by May all the treated plots had improved relatively to the controls. The improvement of the combine-drilled plots was much less than that of the seed-dressing plots.

The plots were scored for visual appearance on 19th April, a complete failure scoring 0 and a good crop scoring 10; these observations are included with the results in Table V.

The mean numbers of plants per unit sample showed no evidence of the chemicals affecting germination.

There was a significant reduction in percentage attacked shoots on all the treated plots compared with the controls. It seems that on the treated plots the plants were able to grow away from the attack and produce a greater number of new shoots than on the control plots. It is also clear that there were marked differences in the percentage of live larvae. The seed dressings were more effective in killing larvae than the combine-drilling treatment, the effects of which were

proportional to their concentrations. The mode of action of combine-drilled dieldrin was apparently similar to dieldrin seed dressings in killing the larvae inside the plant. There were, however, no significant differences in yield other than between treated and untreated.

TABLE V.

Comparison of combine-drilled dieldrin with seed dressings.

Treatment	Score for visual appearance (19.iv)	Plants/sample (13.iv)	Attacked shoots (13.iv)		Percentage live larvae (13.iv)	Yield (cwt./acre)
			%	Angles		
Combine-drilled						
Dieldrin 1 lb./acre	6.1	43	34	36	54	52.4
Dieldrin 4 lb./acre	6.8	41	35	36	21	51.8
Seed dressings						
Dieldrin, 50% ..	7.1	40	26	30	6	50.8
Aldrin, 40% ..	6.4	38	34	35	4	48.4
Control	3.9	40	63	53	88	26.7
S.E. of treatment means		±1.8		±3.8		±1.8

Experiment 5.

In this experiment, a 7 × 4 randomised block, it was planned to compare 40 per cent. aldrin and dieldrin seed dressings with much higher concentrations, but in the event it proved impossible to obtain over 50 per cent. at that time. In subsequent years, up to 70 per cent. became available. A completely untreated control was included as well as the usual organo-mercurial control to see if the latter had any insecticidal effect. The treatments are listed with the results in Table VI and all were applied at 3 oz./bushel.

TABLE VI.

Comparison of aldrin, dieldrin and γ BHC as seed dressings.

	Plants/sample (9.iv)	Attacked shoots (9.iv)		Percentage live larvae (9.iv)	Yield (lb./plot)
		%	Angles		
Aldrin, 40%	29	8	16	0	16.7
" 50%	29	14	22	0	17.0
Dieldrin, 40%	29	12	20	0	17.1
" 50%	29	14	22	0	16.7
γ BHC, 40%	31	1	4	67 (2 out of 3)	16.8
Control (O.M.)	36	16	23	91	15.7
Control (nil)	27	41	39	89	13.9
S.E. of treatment means	±2.9		±2.4		±0.50

There were few visual differences in this trial between the plots, as the larval population was much lower than usual. This was expected from the egg count, and a lower seeding rate and wider spacing between the rows than is normal were chosen in order to concentrate the larval attack on the small number of plants.

The number of plants per sample is higher for organo-mercurial than for all

other treatments though no differences are significant. All insecticides reduced the percentage of attacked shoots, as compared with organo-mercurial seed dressing, but only the difference for γ BHC was significant. The aldrin and dieldrin seed dressings reduced but did not completely prevent the entry of the larvae, but none of these larvae survived. The γ BHC seed dressing, on the other hand, almost completely prevented entry of the larvae, but in the three shoots which were attacked in the samples subjected to detailed examination, two living larvae were found.

It is difficult to explain the high percentage of attacked shoots on the *nil* plots as compared with the organo-mercurial plots. This difference was, however, at least partly due to the lower emergence on the undressed plots where the absolute number of attacked shoots was lower than on the organo-mercurial plots.

The yields of the treated plots were all higher than that of the organo-mercurial plots, but the differences were small and scarcely significant, though their yields were significantly higher than that of the *nil* plots. The small difference in yields is accounted for by the light attack.

Experiments 6a to 6h.

Experiments 3, 4 and 5, carried out in the 1954-55 season, showed that seed dressings of aldrin, dieldrin and γ BHC could, under certain conditions greatly improve crops subjected to wheat bulb fly attack. In order to test their effectiveness over a wide range of soils and sowing dates, it was decided to lay down a series of trials in 1955-56 (Expt. 6a-h) consisting of unreplicated strips, each one drill width and never less than 1/20 acre in area. The treatments were 40 per cent. dieldrin seed dressing (D), 40 per cent. γ BHC seed dressing (B) and control (O.M.). The two insecticides were applied at 3 oz./bushel.

Aldrin seed dressing was omitted for simplicity, as its performance seemed to be very similar to that of dieldrin. Hybrid 46 wheat was used at all sites except 6b which was sown with Cappelle-Desprez. The results, arranged in order of sowing date, are summarised in Table VII.

TABLE VII.

Results of 3-strip trials; Expt. 6a-h.

Site	Sowing date	Soil type	No. plants per sample			Percentage shoots attacked			Percentage live larvae			Yield (cwt./acre)		
			O.M.	B	D	O.M.	B	D	O.M.	B	D	O.M.	B	D
a	15.xi	Clay loam	30	32	33	35	17	23	96	75	18	22	18	27
b	24.xi	" "	21	15	21	76	19	36	89	89	0	7	25	22
c	28.xi	" "	28	29	31	20	17	19	91	50	40	26	23	29
d	1.xii	" "	31	26	30	74	23	22	85	74	12	16	34	35
e	2.xii	Loamy peat	26	29	20	68	42	55	81	64	37	9	29	25
f	30.xii	" "	36	33	27	51	35	41	92	67	0	21	29	30
g	4.i	Sandy loam	24	33	25	75	7	40	94	40	0	3	29	23
h	18.i	Peaty loam	26	29	24	54	28	50	89	72	23	5	19	20
		Mean	28	29	27	57	24	36	90	66	16	14	26	26

Although, as one would expect, there is much variation in the figures in Table VII from different sites, an examination of the means gives the following picture which is substantiated by an inspection of the results for the individual sites. Seed dressings in general have had little effect on initial plant stand though on

sites (b) and (d) there was a noticeable reduction with γ BHC. In a similar series of trials in the East Midlands (R. Gair, *in litt.*) this effect was very marked and occasionally amounted to a 50-60 per cent. reduction of stand. There was no apparent reason for this, nor have any reductions of this order been noted with γ BHC since. It should be noted that 3 oz./bushel is higher than the standard 2 oz. rate. Reductions of stand with dieldrin are noticeable on the later sowings and can be attributed to plants being attacked before they had reached the surface. In one trial on a light peat soil, which was not carried through to completion and is not recorded here, the dieldrin treatment was associated with a marked reduction in plant stand and this again appears to be an isolated and inexplicable occurrence.

Both treatments have reduced the percentage attacked shoots compared with the controls, the reduction with γ BHC being greater than that with dieldrin, except at site (d). Within the two-month range of sowing date there was no suggestion of increased effect with later sowing but it should be noted that the first four sowing dates were on mineral soils and there was a very great reduction with γ BHC on the late-sown site (g) also on a mineral soil. It seems likely that the seed dressings have been less effective on the peaty soils; compare for example the results of (f) and (g).

The percentage of live larvae has been reduced slightly by γ BHC and greatly by dieldrin. Again there appears to be no correlation with sowing date. The final column of yield shows that there is little to choose between γ BHC and dieldrin and, except on the two sites (a) and (c) where the percentage attack on the control plots was low, there is a very marked and consistent increase in yield with both treatments.

Experiments 7a and 7b.

In experiments 7a and 7b, a further comparison was made on two sites between seed dressings and combine-drilling. The seed dressings were 40 per cent. aldrin, 40 per cent. γ BHC and 70 per cent. dieldrin; 4 per cent. aldrin and 4 per cent. dieldrin (both in gypsum) were combine-drilled at 1 cwt. per acre so that about 4 lb. active ingredient was applied per acre. An additional similar plot to the controls was sprayed in mid-March with 0.05 per cent. parathion (Bardner, 1959) at 100 gal./acre.

Again, visual results confirmed the initial protectant effect of the γ BHC treatment which was later caught up by the aldrin and dieldrin seed dressings. On both sites the combine-drilled treatments looked more vigorous than any other treatments from the start, and up to May or June the plants were taller than on the other treatments. At Stonea the control plots were thinned but were not failures as they were at Warmington.

The results in Table VIII (excluding the spray) show that all treatments have again reduced the percentage of shoots attacked. The difference in the treated plots between the two dates of examination is very marked at Stonea and reflects the tillering of the plant and the continuing or increasing effect of insecticide. At the first count, the γ BHC seed dressing and both the combine-drilled treatments appreciably reduced attack. At Stonea, the percentage of live larvae was significantly reduced by all treatments containing aldrin or dieldrin. As the only examination at Warmington was made in April, comparable figures are not available. In both trials, all insecticide treatments except parathion spraying greatly reduced the percentage shoot attack in April and significantly increased yields compared with the control. At Stonea, yield differences in all treatments except the parathion spraying and the control (O.M.) were slight and mostly not significant, but at Warmington the yields from the γ BHC seed-dressing plots were significantly lower than both combine-drilled treatments and the aldrin seed dressing. There was no suggestion that 70 per cent. dieldrin was more effective than 40 per cent. aldrin, and it also seemed clear that there was little to choose between

aldrin and dieldrin when combine-drilled. Even if the yield increases of combine-drilling over seed dressing proved to be consistent, they seemed unlikely to compensate for the greater cost and possible adverse effects on beneficial soil organisms.

TABLE VIII.

Comparing seed dressings and combine-drilling.

Treatment	Plants/ sample (19.iii)	(a) Stonea					(b) Warmington		
		Attacked shoots			Percent- age live larvae (19.iii)	Yield (cwt./ acre)	Plants/ sample (7.iii)	Attacked shoots (19.iv) %	Yield (cwt./ acre)
		19.iii		19.iv					
		%	Angles	%					
Combine-drilling									
Aldrin 4 lb./acre	45	32	34	7	31	38.1	23	22	41.0
Dieldrin 4 lb./acre	45	28	31	10	50	36.4	24	26	41.3
Seed dressings									
Aldrin, 40% (3 oz./bushel)	40	46	43	11	39	37.9	22	46	38.7
Dieldrin, 70% " "	35	41	40	12	53	37.9	21	34	35.8
γ BHC, 40% " "	36	27	31	9	81	36.4	21	48	34.0
O.M. (2 oz./bushel)	40	48	44	51	89	31.7	23	85	13.0
Spray									
Parathion, 0.05%, at 100 gal./acre	—	—	—	33	—	31.5	—	82	14.1
S.E. of treatment means	±2.5		±3.2			±0.54	±0.3		±1.36

Experiment 8.

In Experiment 8, dressings containing 70 per cent. of aldrin, dieldrin, DDT, γ BHC and endrin were compared with one containing 20 per cent. parathion (Bardner, 1958). Again a *nil* control was included. This experiment was on small, hand-drilled plots with wide row spaces and a low seed rate.

Despite the low infestation, visual differences between insecticidal treatment and controls were marked, especially in the early stages of attack, and the γ BHC, dieldrin, aldrin and parathion treatments all stood out as being better than endrin and DDT. The results, recorded in Table IX, revealed, however, that once again no other treatment reduced the primary attack to the same extent as γ BHC, though parathion was better than the remainder. Neither γ BHC nor DDT had affected larvae inside the plant, and aldrin and dieldrin were much more effective in this respect than endrin and parathion. All treatments gave significantly higher yields than the two controls, but there were no significant differences in yield between treatments or between the two controls. It is interesting, however, to note that, as in Expt. 5 (Table VI), there was some evidence that the mercury control was rather better than the *nil* control.

Experiment 9.

Further experiments on different seed dressings and comparison of these with combine-drilled aldrin were carried out between 1956 and 1960 and will be reported elsewhere. During this period, chemical treatments for wheat bulb fly became commercially available and were used extensively in areas where this insect was

often a problem. Their enhanced effectiveness on late-sown crops led to the question as to whether it was better to reduce damage by sowing early without an insecticide or by sowing later with an insecticide. Experiment 9 was laid down in 1959 to compare 40 per cent. γ BHC and 60 per cent. dieldrin and an organo-mercurial seed dressing for sowing dates in October, November and December. The nine treatments were randomised within four blocks.

TABLE IX.

Comparisons of various seed dressings.

Treatment	Plants/ sample (3.iv)	Shoots attacked			Live larvae			Yield (lb./plot)
		Mar.		Apr. %	Mar. %	Apr. %	Apr. No./ sample	
		%	Angles					
γBHC, 70%	29	11	19	3.1	88.9	66.7	0.5	12.8
Dieldrin, 70%	29	37	38	9.4	0	0	0	12.8
Aldrin, 70%	32	36	37	10.0	2.0	12.5	0.25	11.8
Endrin, 70%	27	30	33	11.4	45.4	82.3	3.5	11.8
DDT, 70%	30	40	39	34.0	87.5	93.8	7.5	10.7
Parathion, 20%	32	20	26	7.6	30.0	77.8	1.8	11.9
O.M., 0.8%	30	47	43	58.4	93.5	80.0	6.0	8.4
Nil	29	58	50	69.3	92.0	73.9	8.5	8.2
S.E. of treatment means ..			±2.3				±2.5	±0.56

Unfortunately a number of different factors operated in association with the sowing date. Soil conditions were unusually dry in October 1959 and germination of the early-sown plots was slow; when the seedlings did emerge they were severely grazed by rabbits or hares to such an extent as to delay development (*cf.* Gough, 1955). By the time of the November sowing, rain had fallen and these plots germinated quickly and there was little grazing. The December-sown plots were slightly thinned by birds. The results of this experiment are shown in Table X.

TABLE X.

Comparing seed dressings at three sowing dates.

Sowing date	Seed dressing	Plants/ sample (4.ii)	Plants/ sample (31.iii)	Attacked shoots (31.iii.) % Angles		Live larvae (31.iii) % No./ sample		Yield (cwt./ acre)
23.x	γ BHC, 40% ..	37	33	31	34	83	14	34.3
	Dieldrin, 60% ..	35	31	31	34	66	13	34.1
	O.M.	36	32	39	39	95	17	31.4
17.xi	γ BHC, 40% ..	31	29	21	27	78	11	39.6
	Dieldrin 60% ..	29	30	13	22	37	4	42.2
	O.M.	31	30	24	30	95	12	39.5
17.xii	γ BHC, 40% ..	28	25	17	24	81	5	31.1
	Dieldrin, 60% ..	30	27	9	17	9	0.4	35.4
	O.M.	27	21	25	31	90	6	23.5
S.E. of treatment means ..		± 1.56	± 1.45	± 1.81		± 1.75		± 0.93

In view of the factors affecting the October-sown plots it is difficult to draw conclusions from these as the delay in development may have had effects similar to a much later sowing date. This is suggested by the fact that the number of shoots per plant on 31st March for October sowing is intermediate between the numbers for the November and December sowings (Table I). It is interesting to note in the October-sown series the slight yield increase of both treated plots over the controls. The efficiency of both insecticides in reducing larval entry is enhanced with later sowing though, surprisingly, dieldrin is better than γ BHC in the November and December sowings and equal to it in October. The increasing effectiveness of dieldrin in killing larvae in the plants as the sowing date gets later is very marked. It is clear that, on peaty soils, November sowing without an insecticide is better than December sowing with an insecticide.

Reference to the number of larvae per acre for this trial in Table I shows a decrease with time in the control plots. To some extent this is due to the reduced number of shoots with a later sowing date, as already noted in Experiment 3, but this is not a full explanation. In a preliminary sampling of about 100 plants from each of the October- and November-sown control plots at the end of January, it was clear (Table XI) that there was a heavier attack on the October-sown plots. A week later the results of a standard count on all replicates showed that December plots were much less infested than the other two, and in the main count on 31st March there is a marked gradation of infestation with time of sowing.

TABLE XI.

Relation of sowing dates to attack on control plots.

Sowing date	Percentage attacked plants on various sampling dates				Shoots/plant (4.ii)
	27.i	4.ii	25.ii	31.iii	
October	8.4	30.7	—	75	2.3
November	1.0	34.5	—	59	2.0
December	—	13.4	32	53	1.0

It seems unlikely that many larvae would have hatched before the germination of the December-sown wheat, which is attractive to young larvae (Stokes, 1956), but if the attractiveness of wheat plants (Long, 1958) increased with age, fewer larvae would find the younger plants. It is possible that slight differences in sowing depth at the different dates might affect the larval response but there were certainly no obvious differences in drilling depths.

Discussion.

Our later field experiments were planned in conjunction with laboratory and field work carried out at Rothamsted Experimental Station. Experiments designed to study the effect of sowing date indicate that insecticidal seed dressings are more effective in controlling wheat bulb fly the later the wheat is sown. This agrees with the results of Way (1959), who also showed that at all sowing dates insecticidal effect was greater with wheat sown $\frac{1}{2}$ in. deep as compared with wheat sown 3 in. deep. Way has also discussed the relative importance of contact and systemic action for both γ BHC and dieldrin, in relation to the entry of larvae, and the results of our experiments are consistent with his conclusions.

It is impracticable to study the effect of soil type in field experiments and in the one season when several single experiments were done on different soil types the sowing dates were also variable. However, the impression has been gained over the whole series that better results have been obtained with insecticides on mineral soils than on organic peaty soils, which agrees with experience with other pests.

Bardner (1958, 1959) tested many different chemicals as seed dressings at various rates, but all the materials which he found effective (except for heptachlor, results for which will be described in a later paper) other than γ BHC, aldrin and dieldrin had practical disadvantages of formulation or high mammalian toxicity. Our results show clearly that dry seed dressings containing at least 40 per cent. of one of these three insecticides and applied at 2-3 oz./bushel always reduced damage, the extent depending on sowing date and possibly soil type, and in the presence of moderate to high infestations greatly increased yield. Very much greater quantities of insecticide per acre combine-drilled were needed to obtain comparable improvements. Increasing the concentration of insecticide above 40 per cent. in the seed dressing did not, as a rule, give significant improvements.

Summary.

Gamma BHC, aldrin, dieldrin, endrin, DDT and parathion have been tested as seed dressings in field experiments for the control of wheat bulb fly, *Leptohylemyia coarctata* (Fall.), in eastern England. The concentrations ranged from 20 to 70 per cent. and the dressings were applied at 2-3 oz./bushel.

At concentrations of 40 per cent. or over, the first three materials appreciably reduced attack and at high infestations increased yield; within the period October to January their effectiveness increased the later the wheat was sown. There was evidence that they were less effective on highly organic soils. The other materials were either less effective or had other disadvantages.

Seed dressings of γ BHC usually reduced the number of larvae entering the plant but had little effect on larvae which succeeded in entering. Aldrin and dieldrin and probably most other materials usually had less effect on the entry of larvae but were very effective in killing larvae after entry so that the plant could subsequently recover.

Combine-drilling of aldrin or dieldrin affected the larvae in the same way as seed dressings; in one season on a peaty soil seed dressings were more effective than combine-drilling, but in another season on a mineral soil seed dressings were slightly less effective than combine-drilling. However, it was clear that with combine-drilling very much higher quantities of insecticide per acre were needed to achieve a comparable result. Both aldrin and dieldrin gave similar results.

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FIELD INFESTATION OF COWPEA (*VIGNA UNGUICULATA*) PODS BY BEETLES OF THE FAMILIES BRUCHIDAE AND CURCULIONIDAE IN NORTHERN NIGERIA.

By P. F. PREVETT, B.Sc., A.R.C.S., D.I.C.

*West African Stored Products Research Unit,
Kano, Northern Nigeria.*

Stored cowpeas (*Vigna unguiculata*) suffer severe losses as a result of infestation by Bruchid beetles in all parts of Nigeria. In the Northern Region, in the vicinity of Kano, the predominant species is *Callosobruchus maculatus* (F.), which is evident in new-crop cowpeas as soon as they become available for sale in the markets. A second species, *Bruchidius atrolineatus* (Pic), is less common and appears to be more prevalent during the early months of storage. A weevil, *Piezotrachelus varius* (Wagn.) (CURCULIONIDAE, APIONINAE), is sometimes found in new-crop cowpeas. 1/3

The present study was carried out in order to assess both the importance of field infestation and the relative status of the pest species involved.

Methods.

Cowpeas are seldom grown in Kano Province as a pure-stand crop, but are normally intersown with groundnuts, sorghum or millet. Therefore, in order that the necessary observations might be conveniently carried out, a half-acre plot at the Kano Farm Centre, Northern Regional Ministry of Agriculture, was sown with cowpeas, using the local white-seeded variety. One half of this plot was sown on the 25th June and the other half on the 20th August 1960. The purpose of the two sowing dates, one early and the other a little later than that normally adopted by farmers, was to investigate any difference in degree of infestation of pods at different ripening dates.

Consideration was also given to three farmers' plots in the vicinity of a small village, Tarauni, on the outskirts of Kano, and to pods from a 4- to 5-acre stand of cowpeas at the Agricultural Station at Gaya, approximately 35 miles east of Kano. In the case of the plot at the Kano Farm Centre, pods were harvested at intervals of, usually, one week, whilst only one complete harvest (the normal farmers' practice) was made from all other plots. After harvest, pods were examined at intervals of one day (with a few exceptions, see figs. 2-5) for emergence of insects.

Pods on the Kano Farm Centre and farmers' plots were examined during pod formation for egg-laying by Bruchids.

Finally, after all insects had emerged, the pods from two harvest dates of the plot at the Kano Farm Centre, and from two of the farmers' plots, were shelled in order to assess the degree of damage resulting from the infestation recorded.

Field observations during pod growth.

A number of species of Lepidoptera and Hemiptera were observed to be attacking the young green pods, and, of these, two species of Hemiptera (Family COREIDAE) caused by far the greatest amount of damage. The species concerned, in order of importance were *Acanthomia tomentosicollis* (Stål) and *Anoplocnemis curvipes* (F.). The result of this infestation, which was particularly prevalent on

the Kano Farm Centre plot (predominantly on the first sowing), was premature shrivelling and drying of the pods.

On a number of occasions during early October, adults of *P. varium* were observed on young green pods on the early-sown section of the plot.

Bruchid eggs were very seldom observed to have been laid on young green pods. Oviposition did not usually commence until the pods were fully formed and light green in colour, *i.e.*, just prior to yellowing. Eggs of *B. atrolineatus* were readily distinguishable from those of *C. maculatus* (see fig. 1) and were almost

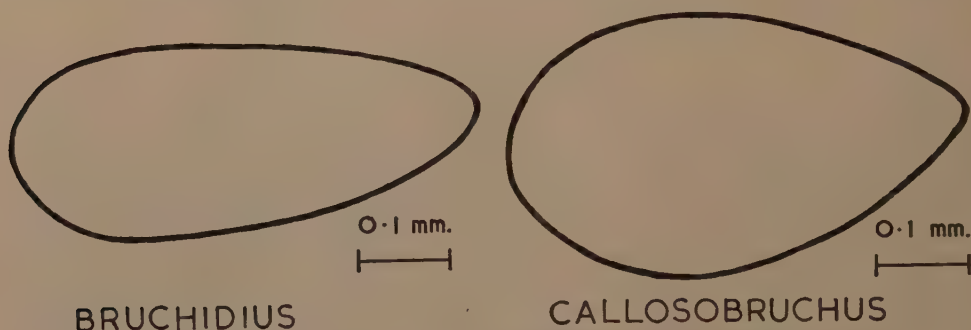


Fig. 1.—Outlines of the eggs of *Bruchidius atrolineatus* and *Callosobruchus maculatus*.

invariably laid upon or beside the septum of the pod. Eggs of *C. maculatus* were laid on the sides of the pod, usually on that side less affected by direct sunlight.

On the 31st October, a comprehensive assessment of egg numbers was made on the second sowing at the Kano Farm Centre plot. Two hundred and sixteen pods were found bearing Bruchid eggs, some of them bearing eggs of both species. A high level of egg-parasitism by *Trichogramma* sp. was observed, eggs in an advanced state of parasitism being black in colour. The data recorded are given in Table I; all eggs from translucent white to yellow in colour were recorded as 'freshly laid' and subsequent figures of Bruchid emergence show that a large proportion of these, particularly in the case of *C. maculatus*, either were, or became, parasitised.

The oviposition habits of *P. varium* are described by Phelps (1956).

TABLE I.

Numbers of Bruchid eggs recorded at Kano Farm Centre plot.

Bruchid species	No. of pods with eggs	No. of eggs freshly laid	No. of eggs parasitised	No. of eggs hatched	No. of eggs empty (2)	Mean no. of eggs per pod	Highest no. of eggs on one pod	Total no. of eggs
<i>B. atrolineatus</i>	140	273 (46.3) (1)	262 (44.3)	55 (9.4)	0	4.2	16	590
<i>C. maculatus</i>	182	934 (68.5)	432 (31)	1	5	7.5	55	1371

(1) Numbers in parentheses are percentages.

(2) Egg-shell empty: contents presumably sucked out by predator.

Emergence of insects from harvested pods.

The total numbers of insects emerging from the various batches of pods considered are summarised in Table II.

Figures recorded from regular harvests from the Kano Farm Centre plot indicate that numbers of *P. varium* and Hymenopterous parasites* were initially at a high level and subsequently declined, suggesting an association between these species.

TABLE II.
Emergence of insects from pods.

Plot location		Date of harvest	No. of pods	No. of <i>P. variump</i>	No. of parasites	No. of <i>B. atrolineatus</i>			No. of <i>C. maculatus</i>		
						♂	♀	Total	♂	♀	Total
Kano Farm Centre	Early	17.x.60	180	242	373	11	13	24	2	0	2
		24.x.60	500	143	169	16	15	31	0	1	1
		12.xi.60	250	21	86	13	10	23	5	2	7
	Late	12.xi.60	1000	582	555	88	104	192	22	23	45
		19.xi.60	500	212	184	15	12	27	24	22	46
		26.xi.60	500	31	27	13	19	32	38	36	74 (1)
		3.xii.60	500	15	62	16	9	25	26	19	45
		10.xii.60	500	54	53	37	30	67	32	34	66
		17.xii.60	500	14	3	20	27	47	35	23	58 (1)
		24.xii.60	400	0	6	11	25	36	15	12	27
		31.xii.60	300	0	25	24	26	50	39	34	73
	Gaya		21.xi.60	500	124	79	28	35	63	158	164
Farmers' plots	1	8.xi.60	500	780	120	208	180	388	11	12	23
	2	8.xi.60	500	579	225	204	217	421	18	18	36
	3	9.xi.60	500	427	197	89	105	194	15	10	25
Totals			7130	3224	2164	793	827	1620	440	410	850

(1) It is probable that this total is made up of $F_1 + F_2$.

(2) Total made up of $F_1 + F_2$ (see fig. 5).

Numbers of *B. atrolineatus* were also initially high and declined later. Infestation by *C. maculatus* was at a comparatively low level, with only a very occasional appearance of the 'active' form (Caswell, 1960). Infestation by *B. atrolineatus* was at a higher level with a single final harvest from farmers' plots as compared with regular weekly harvests at the Kano Farm Centre. The emergence of *P. varium* and parasites and of *B. atrolineatus* and *C. maculatus* from pods harvested on three occasions from the second part of this plot is shown in figs. 2 and 3, and the average of emergences from pods from the three farmers' plots in fig. 4.

Degree of damage resulting from infestation.

The results of an assessment of damage due to infestation in respect of the seeds from four lots of 500 pods are given in Table III. It was not possible to determine the percentage of pods attacked by the various insect species as a considerable degree of breakage occurred as a result of the daily examinations.

* Material submitted to the Commonwealth Institute of Entomology was reported to contain the following:—EURYTOMIDAE: *Eurytoma* sp.; EUPELMIDAE: *Bruchocida vuilleti* Crwf., *Eupelmus* sp.; PTEROMALIDAE: *Anisopteromalus calandrae* (How.); EULOPHIDAE: two species of *Entedon*, one being represented by a long series.

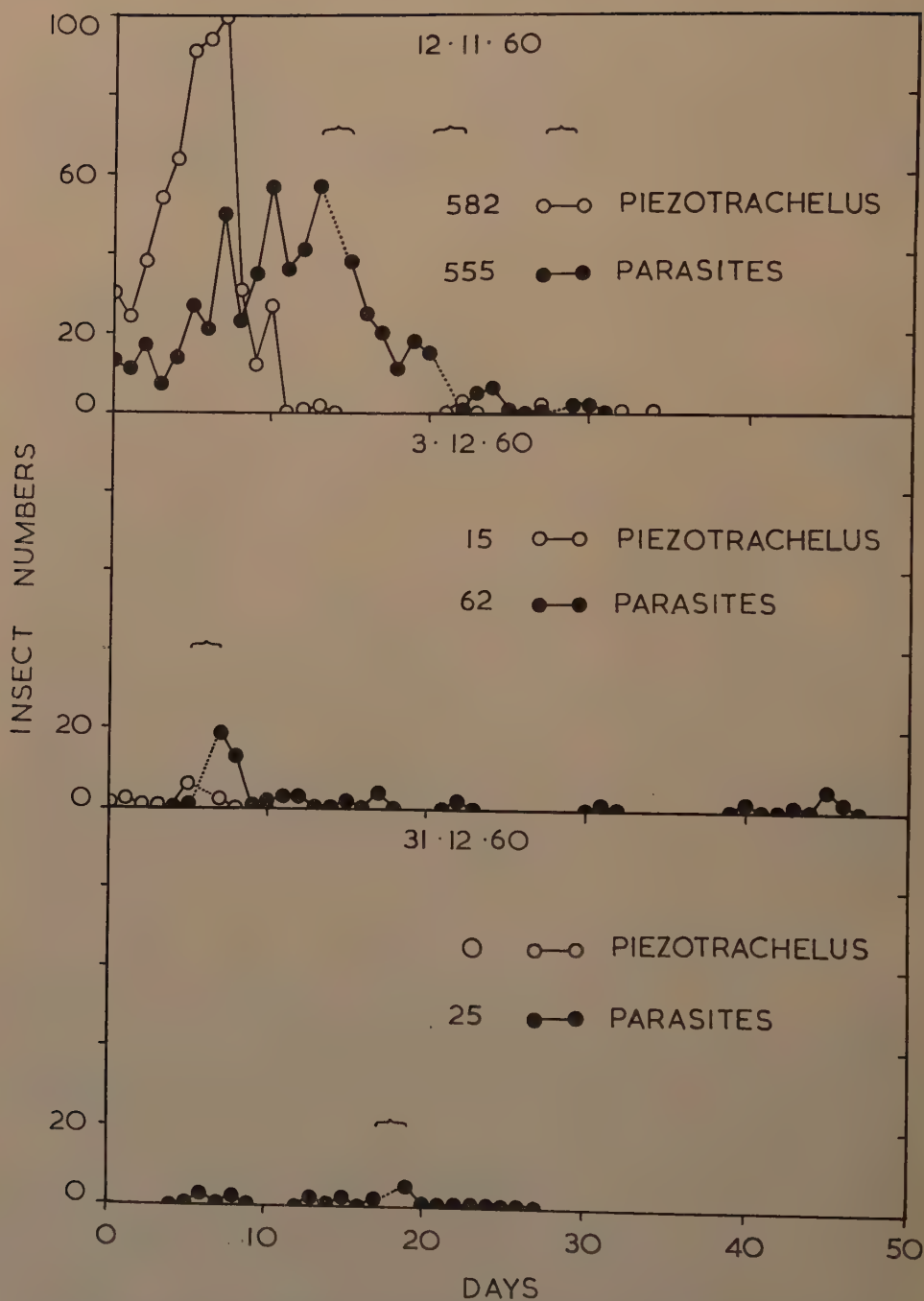


Fig. 2.—Emergence of *Piezotrachelus varius* and Hymenopterous parasites from pods harvested on three occasions from the Kano Farm Centre plot. Brackets denote occasions on which a two-day interval elapsed during pod examination.

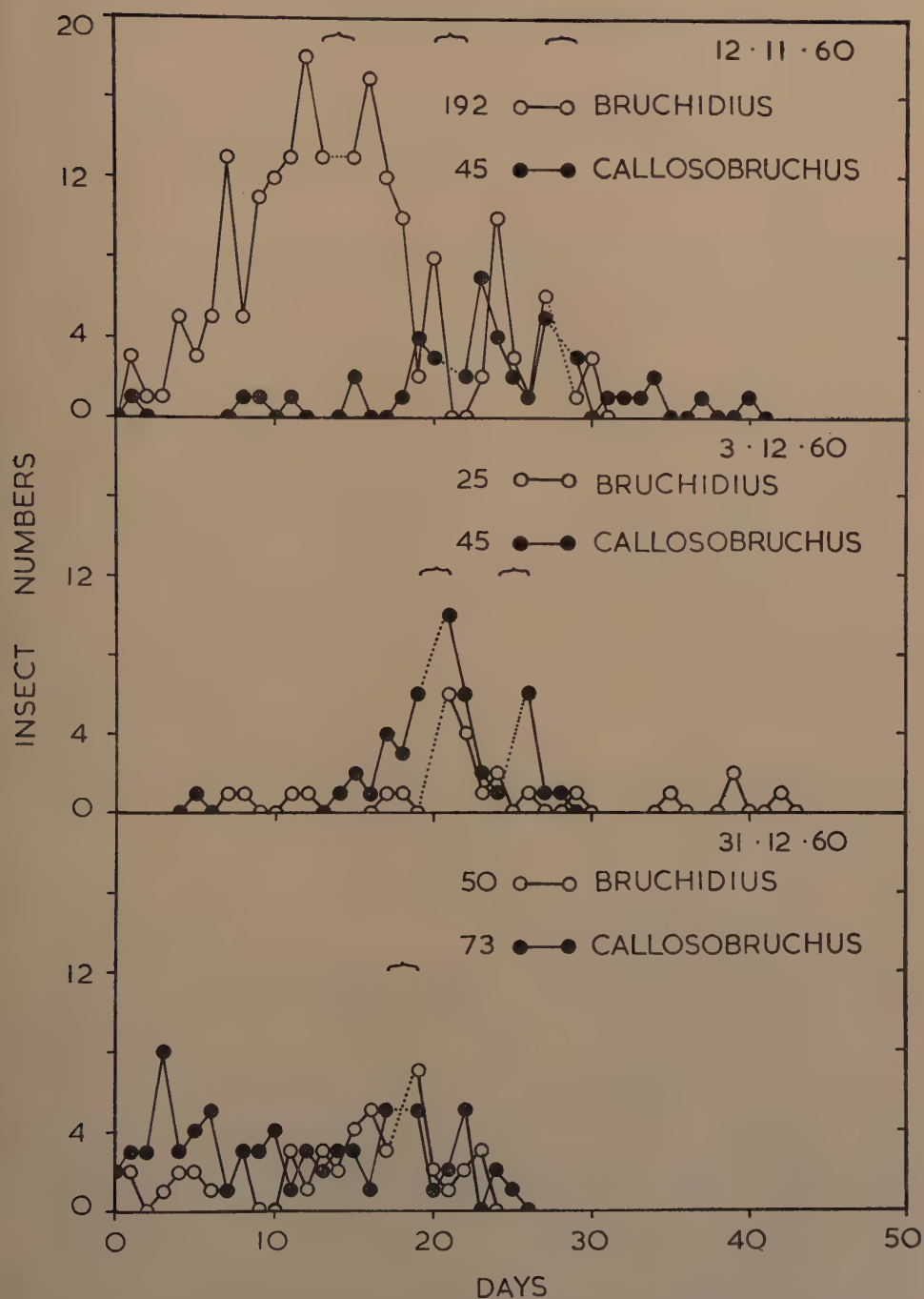


Fig. 3.—Emergence of *Bruchidius atrolineatus* and *Callosobruchus maculatus* from pods harvested on three occasions from the Kano Farm Centre plot (for explanation of brackets, see fig 2).

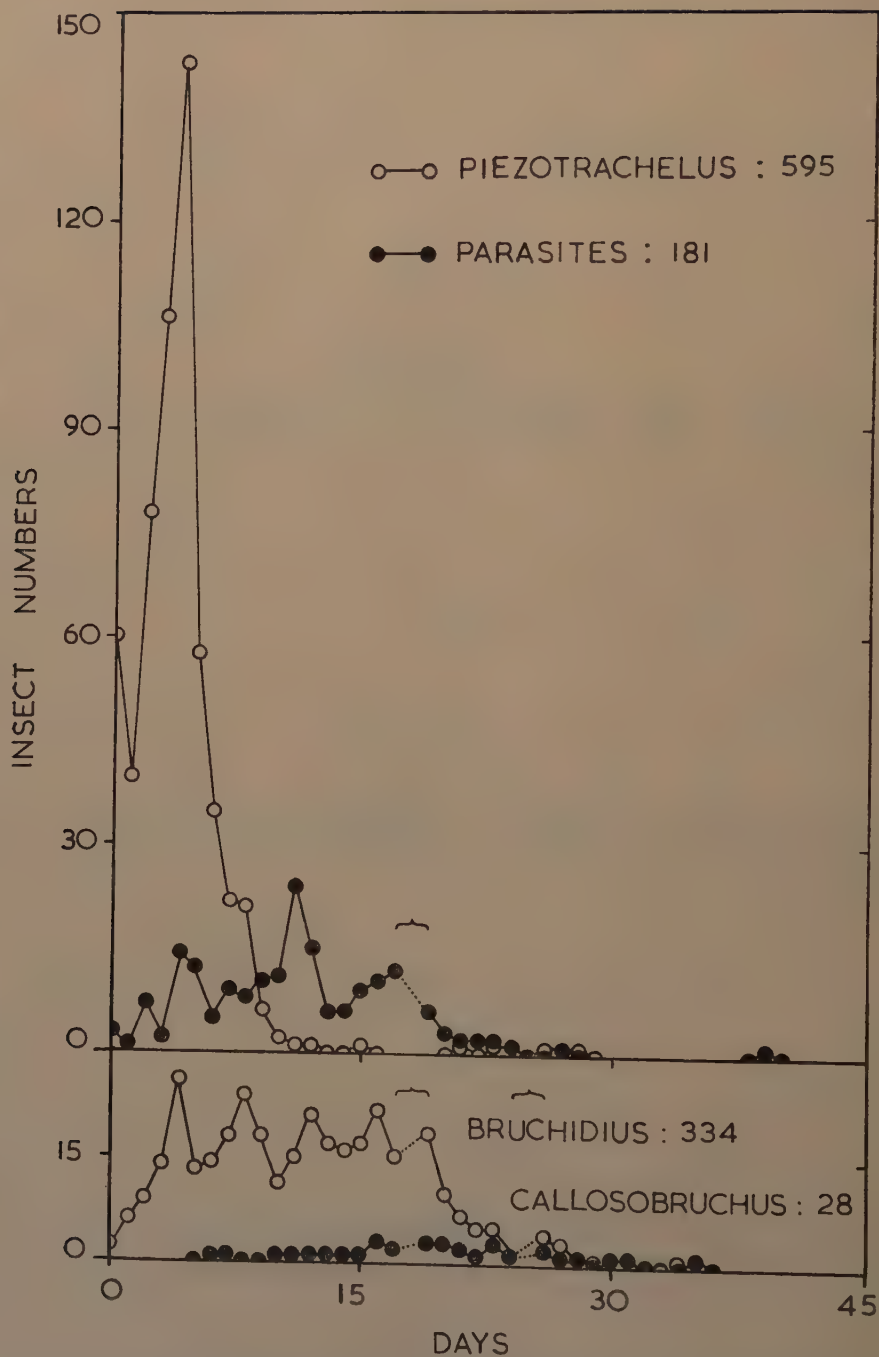


Fig. 4.—Average of emergence of insects from pods harvested from three farmers' plots (for explanation of brackets, see fig. 2).

Regarding seeds, a mean figure of 11 per cent. attack by Bruchids in respect of a single final harvest and 3.5 per cent. in respect of regular weekly harvests were recorded.

The different types of damage were readily distinguishable, Bruchid infestation resulting in the presence of a neat round hole in the seed, whilst infestation by *P. varium* resulted in the disintegration of the portion of the seed affected. /3

Discussion.

It would appear from data given in Table II that a difference does exist between the two sowings of the Kano Farm Centre plot with regard to infestation by Bruchids, though the high level of infestation of the first sowing by Coreid bugs is no doubt partially responsible for this, as the highly shrivelled pods resulting from this infestation are unsuitable for Bruchid development.

Large numbers of *P. varium* were recorded both from the pods harvested from the early sowing and those from the initial harvests from the second sowing. As many as 20 larvae of this weevil have been found within a single seed and the appearance of such heavily infested seeds suggests that the infestation began some time before drying of the seed commenced. This is confirmed by the emergence of weevils immediately after harvest (see fig. 2). Phelps (1956) observed in South Africa that the larvae of *P. varium* each consumed several seeds and it can only be supposed that the infestation to which he refers was of very young pods. The data recorded in the present work, though suggesting that infestation commences late in pod development, does indicate that in Nigeria, as in South Africa, this weevil may be termed a true field pest. It can therefore be inferred that the occasional appearance of *P. varium* in new-crop cowpeas in Nigerian markets is the result of emergence from seeds originating from pods which became dry immediately prior to harvest. /3

As pointed out above (p. 637), it is evident that the majority of Hymenopterous parasites emerged from pods during and immediately following weevil emergence (see figs. 2 & 4), suggesting that they were largely parasitic upon larvae of this species. Phelps (1956) records the emergence of large numbers of parasites from pods infested by *P. varium* and *Apion chirindanum* Wagn. This is confirmed by data given in Table III. Parasites of Bruchids emerge from the dry seeds and emergence may be detected by the presence of a very small hole in the testa (a little less than 1 mm. in diameter compared with the Bruchid emergence hole of approximately 2 mm. diameter). It will be seen that very low numbers of such holes were recorded. /3

Considering the two Bruchid species, a comparison of both the numbers of eggs parasitised and the numbers of eggs hatched (see Table I) would suggest that *B. atrolineatus* commenced oviposition on pods before *C. maculatus*. Data recorded in Table II and represented graphically in figs. 3 and 4 show that *B. atrolineatus* was the predominant species emerging, particularly during the period immediately following harvest. Some of the value of these figures is lost in view of the fact that a proportion of the Bruchids emerging no doubt consisted of the F_2 generation as a result of post-harvest oviposition by beetles emerging from the samples, particularly during the two-day periods indicated in the figures. That such oviposition by *C. maculatus* does occur is shown very clearly in fig. 5, a very large proportion of the beetles emerging belonging to the F_2 generation.

However, the fact that post-harvest oviposition was predominantly by *C. maculatus* tends to confirm the suggestion that this species is primarily a store pest, able to commence its infestation in the field; whilst *B. atrolineatus* is primarily a field pest, able to continue infestation in store to a very limited extent. This is supported by figures of Bruchid emergence from cowpea samples taken regularly from a Kano market during the period February 1960 to February 1961. *B. atrolineatus* was found to emerge in small numbers from samples taken during

TABLE III.
Assessment of damage due to infestation.

Plot	Total no. of seeds	Bruchid damage (1)		³ <i>P. varius</i> damage (1)		Remainder		No. of seeds with Bruchid- parasite emergence holes (3)	Total no. of parasites recorded
		No. of seeds	%	No. of seeds	%	No. of seeds	Per- centage shrivelled (2)	Per- centage un- damaged	
Farmer's 1	4557	513 (411)	11.2	284 (780)	6.2	3750	18.1	64.2	120
Farmer's 2	4528	488 (457)	10.8	326 (579)	7.2	3688	13.7	67.6	225
Kano Farm Centre, harvested 19.xi.60	3452	71 (73)	2.1	123 (212)	3.6	3256	35.5	58.8	184
Kano Farm Centre, harvested 17.xii.60	2268	112 (105)	4.9	18 (14)	0.8	2138	59.2	35.1	3

- (1) Figures in parentheses are numbers of insects recorded. Any discrepancy between these figures and number of seeds damaged indicates some pre-harvest emergence, or more than one beetle per seed, in the case of Bruchids; and more than one insect per seed, in the case of *P. varius*.
 (2) Largely the result of damage by Hemiptera (COREIDAE).
 (3) Seeds in this column have been included in the addition to give totals in column no. 2 with the exception of the fourth line, where the seed had already been included in another column.

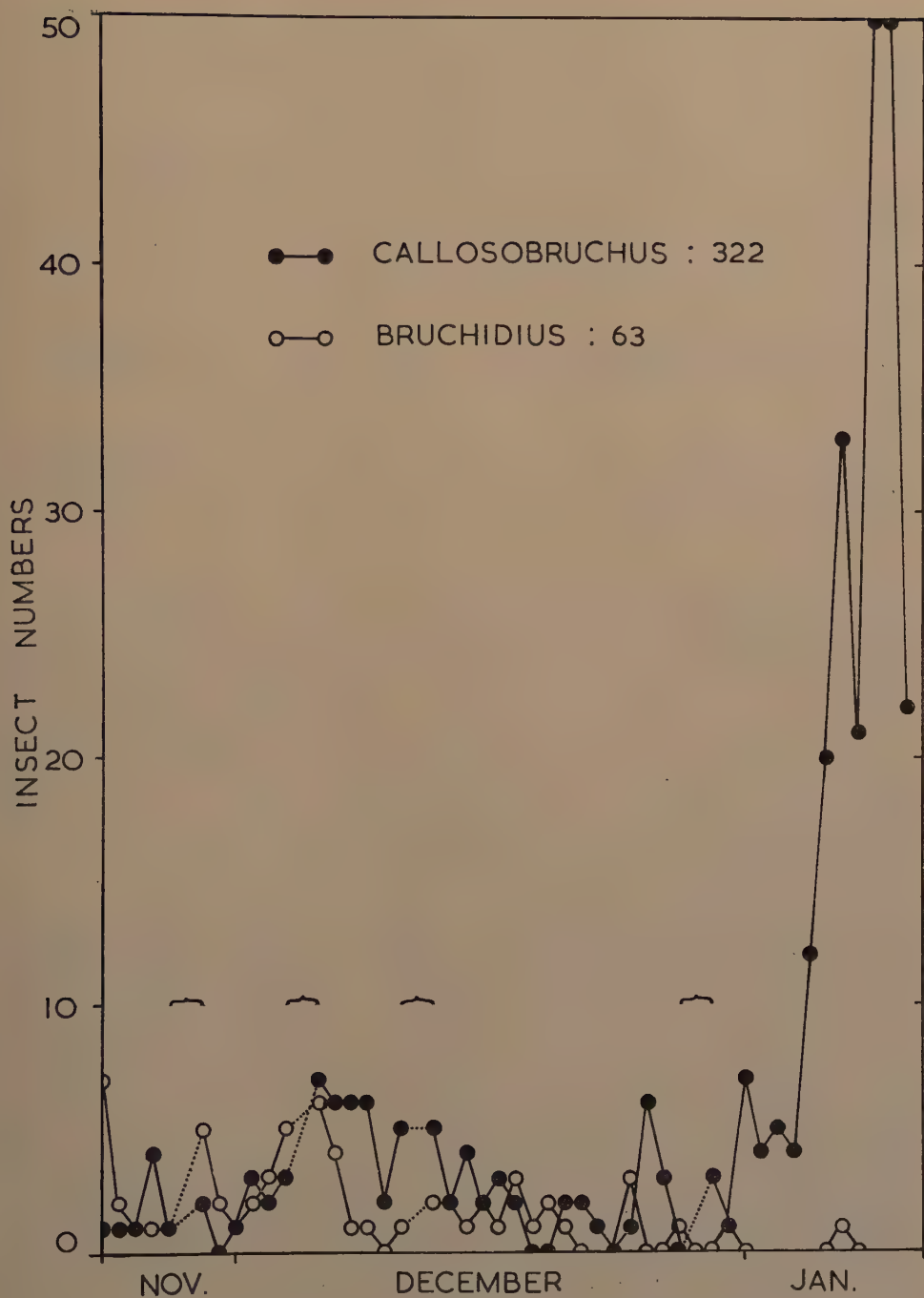


Fig. 5.—Emergence of Bruchids from a sample of pods, illustrating an extreme effect of post-harvest oviposition by *C. maculatus* (for explanation of brackets, see fig. 2).

the period February to April, and again appeared in samples taken from late December onwards, as new-crop cowpeas began to enter the market. No living examples of *B. atrolineatus* were recorded from samples taken between April and December. Infestation by *C. maculatus* was at a high level throughout, until November when a decrease in numbers was observed.

In contrast to the findings of Caswell (1960) in the Western Region of Nigeria, the 'active' form of *C. maculatus* was observed to appear in greatest numbers in market samples taken from June until October, numbers being negligible after the entry of new-crop cowpeas into the market. This suggests that the appearance of the 'active' form in the north of Nigeria may be correlated with poor condition of the food medium, its readiness to fly making it capable of movement to fresh cowpeas, possibly to cowpea pods in the field. Its rare occurrence in the F_1 generation emerging from pods (see p. 637) would tend to confirm this, as activity is no longer an essential requirement for species propagation. Further observations are, however, required before definite conclusions can be drawn, and it is hoped that the data recorded here will promote the making of such observations by future workers.

Phelps & Oosthuizen (1958) record that *C. chinensis* (L.), a serious pest of stored cowpeas in Natal, caused infestation to only 1.9 per cent. of freshly harvested pods, showing that the status of this species is similar to that of *C. maculatus* as interpreted from the present work.

Conclusions.

It is evident that in terms of the ultimate infestation of cowpeas in store the most serious aspect of field infestation is the commencement of attack by the predominant store pest, *C. maculatus*, though a greater degree of damage, as assessed at harvest, results from the infestation of *P. varium* and *B. atrolineatus*.

In the case of the latter two species there is probably little that can be done to obviate this damage in view of the normal method of cultivation. Adults of *P. varium* have been observed to emerge from pods of *Indigofera astragalina* collected in the vicinity of Kano in mid-November 1960, and *B. atrolineatus* has been recorded from pods of a wild species of *Vigna* at Samaru in May 1960 (R. H. Booker, Ministry of Agriculture, Samaru; private communication), and at Kano in March 1961. It would appear, therefore, that an investigation of secondary hosts of these species, followed by their elimination from cowpea-growing areas, might result in a reduction in infestation.

With regard to field infestation by *C. maculatus*, a reduction in the available population of beetles by some form of treatment of cowpeas stored in villages adjacent to cowpea plots would no doubt result in a reduction in pod infestation.

As indicated above, any method of field control would not be practicable, due to the manner of cultivation, and it is evident that consideration will need to be given to some form of control measure suitable for the immediate post-harvest storage period. The normal practice is to store the pods until such time as the seeds are required either for sale or consumption, and there is little doubt that it is during this period that populations of *C. maculatus* build up to the often high level observed on new-crop cowpeas in the markets.

From data recorded above it is clear that a much higher level of infestation by *B. atrolineatus* is present at harvest when only one final harvest is made, compared with regular harvests as the pods become dry, and if the latter system could be adopted by farmers, coupled with a method of control for the harvested pods, a considerable improvement in quality would be apparent.

Summary.

An account is given of a study of infestation of cowpea pods in the field in Northern Nigeria by the weevil, *Piezotrachelus varium* (Wagn.), and the Bruchids,

Bruchidius atrolineatus (Pic) and *Callosobruchus maculatus* (F.). Oviposition by Bruchids did not commence until the pods were fully formed and light green in colour, whilst infestation by *P. varium* originated in the young pods. Observations indicated that a high level of parasitism of Bruchid eggs occurred, but that the majority of Hymenopterous parasites emerging from within the pods were related to *P. varium*.

When pods were harvested at regular intervals, large numbers of *P. varium* emerged from the early harvests only. *B. atrolineatus* was the predominant Bruchid species emerging, particularly from early harvests. In the case of a single final harvest of pods, large numbers of *P. varium*, and a higher level of infestation by *B. atrolineatus* than in pods collected at regular intervals, were recorded.

Shelling of four batches of pods indicated a mean of 11 per cent. of seeds attacked by Bruchids in the case of single final harvests, and a mean of only 3.5 per cent. in the case of regular harvests as pods became dry. It is concluded that a considerable improvement in quality would result from an adoption of the latter method by farmers, provided that some method of control in respect of the harvested pods were introduced.

From the observations made it is concluded that *P. varium* is a true field pest, *B. atrolineatus* primarily a field pest able to continue infestation in store to a very limited extent, and *C. maculatus* primarily a store pest able to commence its infestation in pods in the field and to build up in numbers rapidly during the immediate post-harvest storage period.

The normal manner of cultivation precludes the use of field methods of control at oviposition peaks but observations suggest that elimination of secondary hosts of *P. varium* and *B. atrolineatus* might be worthwhile. A reduction in oviposition by *C. maculatus* by treatment of stored cowpeas in growing areas, and treatment of pods during the immediate post-harvest storage period, should considerably reduce the level of infestation by this species.

Acknowledgements.

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My thanks are due to the Principal Agricultural Officer, Kano Province (Ministry of Agriculture, Northern Region), for his co-operation in organising and placing at my disposal the cowpea plot at the Kano Farm Centre, and for organising regular harvests of pods therefrom; to the Director of the Commonwealth Institute of Entomology for the determination of the Hymenopterous parasites; and to Mr. R. J. Izzard of the British Museum (Natural History) for the determination of *Acanthomia tomentosicollis*.

Messrs. S. C. Morah and J. N. Onyuchi were largely responsible for the daily examination of harvested pods.

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SEASONAL VARIATION IN SIZE AND COLOUR, AND DAILY CHANGES
IN THE DISTRIBUTION OF *GLOSSINA PALLIDIPES* AUST.
IN THE SOUTH BUSOGA FOREST, UGANDA.

By J. P. GLASGOW

East African Trypanosomiasis Research Organization,
Tororo, Uganda.

L.T.

As Buxton (1955) observes (p. 205), authorities are not in agreement on the possibility that some species of *Glossina* migrate with game animals for considerable distances. Swynnerton (1936, pp. 206–207) thought not. "Tsetse, especially males, but females also, will ride on man and animals for a time. A fallacious idea has arisen from this that a fly community will follow a game-herd continuously and so appear wherever the game-herd does—far outside of the normal fly limits. Actually the flies do not live in continual association with their animal hosts." Chorley & Hopkins (1942) took a different view. "It is known that buffalo are a very favourite food of *pallidipes* and that the fly sometimes move out of an area with a herd of buffalo." Jackson (1955) made observations of an area inhabited by *G. morsitans* Westw. in the Northern Province of Tanganyika which was occasionally traversed by herds of buffalo, and he wrote "In these two examples there is, I consider, good evidence against the mass movement of fly communities with herds of buffalo. However, this story is so persistently urged, especially but by no means only in Uganda, that further observations are now being arranged there." The present paper reports these further observations.

Meantime, Morris (1960), working in the same area as myself, has given evidence indicating that swarms of *G. pallidipes* Aust. collect around and follow herds of elephants passing through fly-infested country. His figures are very striking, indicating a trap catch 5–10 times normal in the week in which the elephants passed, although unfortunately they are not subjected to any significance tests, or as Morris himself puts it, they are "not ground to obscurity in the deadly machines of the statisticians."

The place chosen for this investigation was the South Busoga forest in Uganda. This area carried a heavy human population in 1900 and at that time it is presumed that natural vegetation was mostly suppressed except for a fringe of forest along the edge of Lake Victoria. During the great sleeping sickness epidemic in the early years of the century the area was compulsorily evacuated, after which much upgrowth of woody vegetation must have occurred. At some time not exactly known, this secondary vegetation was occupied by *G. pallidipes*, which had previously been absent or very restricted. People began to reoccupy their ancestral lands, but in 1943 an epidemic of *rhodesiense* sleeping sickness transmitted by *G. pallidipes* (MacKichan, 1944) necessitated another evacuation. The site of our intensive observations in 1954–55 was approximately 0°23'N., 33°32'E. The altitude was about 3,800 ft., only a little above the level of Lake Victoria, into which the drainage ran. At Iganga, some 15 miles to the north, the average annual rainfall is 50 in., well distributed, some rain being expected in every month (fig. 2). The country is gently undulating, with broad low ridges and wide flat-bottomed valleys. The valleys, which become waterlogged in wet weather, carry coarse grassland. The sides of the valleys, and any raised, better-drained areas in the valleys, carry evergreen thicket, typically 10–20 ft. high, and extremely dense. The tops of the ridges carry a forest up to 100 ft. high in which the most important

trees are *Chlorophora excelsa* and *Albizia* spp. There is little undergrowth beneath the big trees, and the normal succession appears to be for the forest to replace the thicket, but this is sometimes reversed by elephants knocking down a patch of trees. A detailed record of the vegetation in an area of about 180 acres straddling a valley is given in fig. 1.

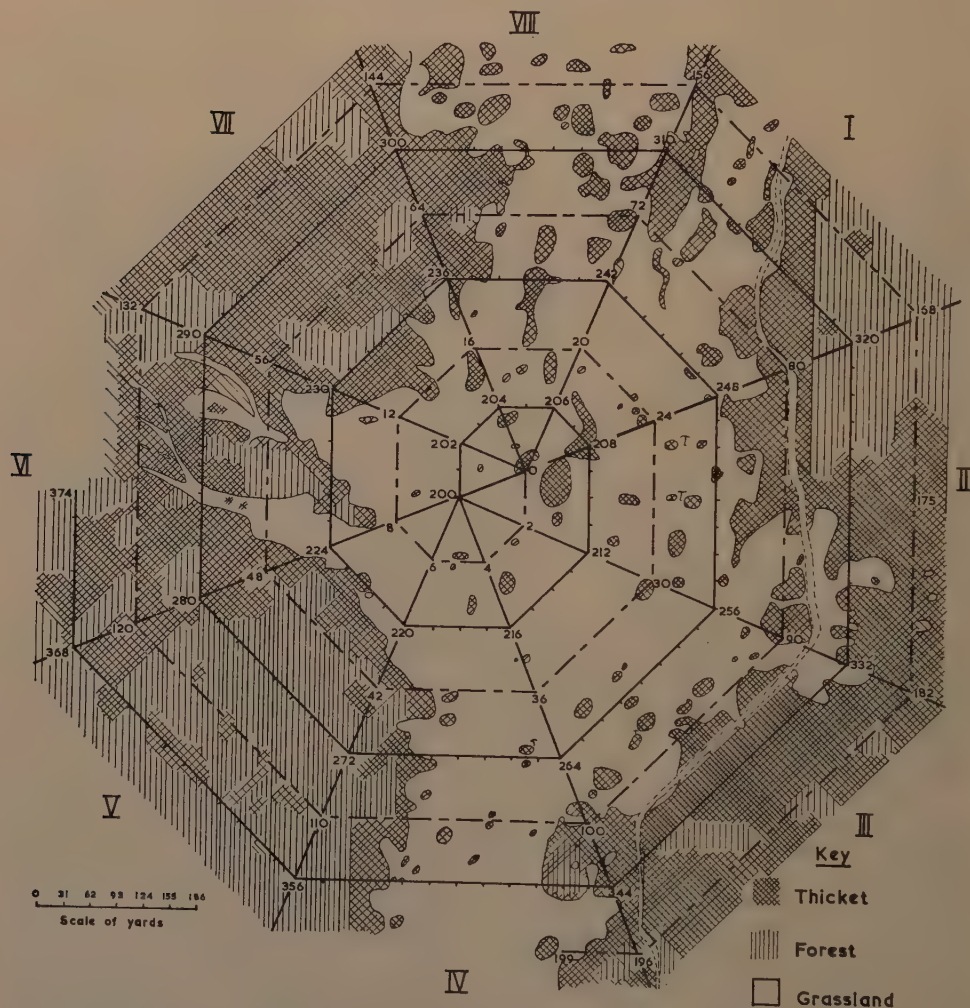


Fig. 1.—Map of the double spiral and the vegetation in its vicinity. The numbers shown (and intermediate numbers not shown) were stamped on metal tags nailed to fixed objects. Note that one path extends from 0 to 199, the other from 200 to 374.

The following animals, or signs of them, were seen frequently and they are regarded as residents: bushbuck (*Tragelaphus scriptus dama*), duiker (*Sylvicapra grimmia*), reedbuck (*Redunca redunca ugandae*), waterbuck (*Kobus defassa ugandae*), bushpig (*Potamochoerus koiropotamus johnstoni*). The names are taken from Swynnerton & Hayman (1951).

Experience in another thicketed area near Lake Victoria (Isherwood & others,

1959) suggests that, among the resident species, bushbuck, a thicket-dwelling animal, is likely to be the most important food source of *G. pallidipes*. No blood-meals were collected during the work described here.

Occasional visits, especially in wet weather, were paid by one or two hippopotamus (*Hippopotamus amphibius*). The area is about four miles from the nearest part of Lake Victoria. In addition there was a herd of elephants (*Loxodonta africana*), thought to number 300, some or all of which visited our area on a number of occasions. Finally, a herd of buffalo (*Syncerus caffer caffer*) sometimes visited the area. No estimate was made of the number of buffalo, but they were probably less numerous than the elephant. The numbers visiting the area varied from one to very many, and sometimes they accompanied the elephants and sometimes they came alone.

Methods.

As shown in fig. 1, two concentric octagonal spiral paths were marked out in the study area. The two paths were 75 yd. apart, and successive whorls of one spiral 150 yd. apart. This distance was selected because earlier experience with *G. pallidipes* in the Lambwe Valley in Kenya had shown that flies marked on one path of a double spiral with 75-yd. separation were recaptured in equal numbers on both paths (Dr. D. L. Johns, personal communication). From this it was inferred that the whole area was being sampled, with no 'dead area' between the paths which did not contribute to the catch. On the other hand, there was (and still is) no available information which would justify increasing the 75-yd. separation. Each path was divided into sections of 31 yd. by means of numbered tags nailed to trees, or posts where no natural support was available. Thirty-one yd. is half 62 yd., the length of the shortest leg of the spiral, which was determined by the distance between whorls. At first the two paths contained, respectively, 175 and 174 sections (joint length 10,819 yd.). After 15th December 1954, the first path (shown as broken line in fig. 1) was extended to 199 sections, making the total joint length 11,563 yd.

The two paths were traversed 4-6 times a week each by a party of three catchers. The parties stopped at each numbered post and caught all the tsetse they could before moving on to the next number. No catching took place between numbers. All flies caught were given an individual mark (Jackson, 1953*a*) and released at once in the same place. A record was kept of the position in which each fly was caught: either 'up', *i.e.*, on the party, or 'down', *i.e.*, on the ground or on vegetation. On the first day, work began at Nos. 0 and 200, on the second day the direction was reversed, work beginning at Nos. 175 and 374, and so on. Work began each morning at about 0830 and finished about noon. Wet- and dry-bulb temperature readings were taken with a whirling psychrometer at the beginning and end of each day's work (except July-September 1954). Mean temperatures, and saturation deficits, calculated from the depression of the wet bulb, are plotted in fig. 2. The rainfall records in fig. 2 are the means of two gauges on Busoga Farms, six miles north of the study area. Records were kept of all animals seen, and of any tracks deemed to be less than 24 hours old.

Collections of *G. pallidipes* were made each month near to but not on the spiral. These were measured, using the length of the 'cutting' edge of the hatchet cell as an index of the size of the fly, as in Buxton (1955); and bearing in mind the recent work of Dr. E. Burt (reported by Jackson, 1954) they were examined in respect of their abdominal pigmentation. It was found that completely pigmented specimens were rather rare, the majority showing some degree of pigment deficiency. Pigment deficiency occurred according to a recognisable pattern, and four colour classes were defined: A, completely pigmented; B, pigment deficiency confined to tergite III; C, pigment deficiency on III and on IV; D, pigment deficiency on III, IV and V.

Each fly was examined alive with a hand lens and assigned to one of these four classes.

Monthly collections of *G. brevipalpis* Newst. were made also for size determination. Although negligible numbers of this species were caught on the spiral, several often came into my car in the place where I used to park it. It was also easy to make large collections of males about sunset. Very rarely, *G. palpalis fuscipes* Newst. was captured on the spiral, and in other places nearby, but appreciable numbers of this species were found only within a hundred yd. or so of the lake.

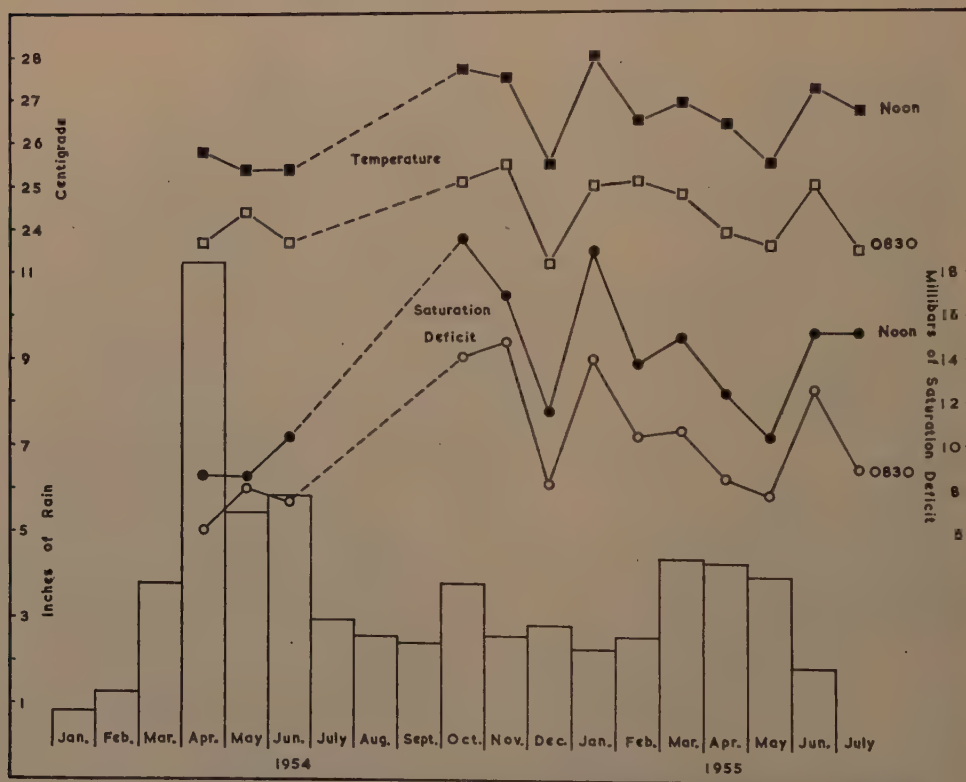


Fig. 2.—The climate. Rainfall from the mean of two gauges on Busoga Farms, temperature and saturation deficit from whirling psychrometer readings at the beginning and end of each day's work on the spirals.

Results.

Seasonal changes in *G. pallidipes* and *G. brevipalpis*.

As shown in fig. 3, seasonal changes occurred in the mean size of *G. pallidipes*. The changes were smaller than those found in Shinyanga by Jackson (1953b), reflecting the more equable climate and better distributed rainfall of Busoga. Also shown in fig. 3 are changes in the mean size of *G. brevipalpis*; I do not know of any previous records of size changes in this species. Chapman (1960) has published records of seasonal variation in size in another member of the group of *G. fusca* (Wlk.), *G. medicorum* Aust. In a climate apparently with greater seasonal differences than Busoga, the range of size in *G. medicorum* was greater

than that recorded here in *G. brevipalpis*. As the dry season advanced, there was a marked change in the relative abundance of the various colour classes of *G. pallidipes*: in June 1954, dark flies were relatively abundant and pale flies rare, the reverse being the case from November onwards (fig. 3). Since these observations were made, Bursell (1960) has studied the abdominal pigmentation of *G. pallidipes* very thoroughly, using a delicate index with 15 colour classes. Unfortunately we found it impossible to equate his system, for use with dry permanent preparations, with mine, for use with living flies. There can be no doubt that basically we were studying the same phenomenon: we discovered independently that males are more responsive to dry conditions than females, and that within one sex larger flies are less pigmented. Study of Bursell's fig. 1 suggests that my Class D would have an index of 10-12; this in turn implies that this class, comprising 50 per cent. of my samples from November to February, had developed in conditions of perhaps 70 per cent. R.H. This would not be harmful for *G. pallidipes* (Bursell, 1958) but would be marginal for *G. brevipalpis*, which presumably has the power of selecting damper breeding spots. Changes in the apparent density of *G. pallidipes* (the mean catch of non-teneral males per 10,000 yd.) are also shown in fig. 3. The catch was high in April 1954 and again in April 1955, and this may be a seasonal effect.

The distribution of G. pallidipes.

In studying the possible effects of buffaloes on *G. morsitans*, Jackson (1955) marked flies daily, until each day's catch included a number of flies marked on previous occasions. Under these conditions the addition of unmarked or the subtraction of marked individuals would be easily detectable. It was proposed to use the same method for studying the effects of large animals on *G. pallidipes* in Busoga, and this was the reason for laying down the spiral. In the event, however, this technique proved impracticable. The daily recaptures of marked flies were too few and sporadic for it to be possible to detect changes in them. Interesting results were, however, obtained by studying the distribution of catches on the spiral.

The total catches of non-teneral males of *G. pallidipes* made at each catching post on the spirals during the seven months January-July 1955 are shown in fig. 4. The catches during April-December 1954, before the first spiral was extended from No. 175 to No. 199, had essentially the same distribution. In an earlier paper (Ford & others, 1959) a method was proposed for assigning thresholds, above which catches would be considered high, and below which they would be considered low. The total catch shown in fig. 1 is 2,584 in 373 places, giving a mean catch of 6.9. Entering Table V of Ford & others at 7, we find thresholds of 13 and 2. In fig. 4, therefore, values of 13 and more are underlined, and values of 2 and less are ringed. Comparing fig. 4 with fig. 1, we see that the underlined numbers tend to occur in the thicket, and the ringed numbers in the grassland or in the high forest, a result in accordance with accepted ideas about *G. pallidipes*.

The data of fig. 4 can be summarised (Table I) by counting the number of posts at which 0, 1, 2, 3 . . . flies were recorded. The resulting distribution, when examined by the methods of Bliss (1953), is found to fall fairly close to a negative binomial. The maximum likelihood value of k is 1.435. The negative binomial distribution calculated from this value of k and the observed mean 6.9276 is shown in the right-hand column of Table I; it differs insignificantly from the observed distribution ($\chi^2=28.72$, $P=0.1$).

It seems to be impossible to avoid using the word "distribution" in two senses in this discussion. The reader must bear in mind that a biologist looking at fig. 4 means by "distribution of *G. pallidipes*" the over-all pattern of catches which may or may not fit the vegetation pattern, whereas a mathematician looking at Table I has quite different concepts in his mind when he thinks of "distribution

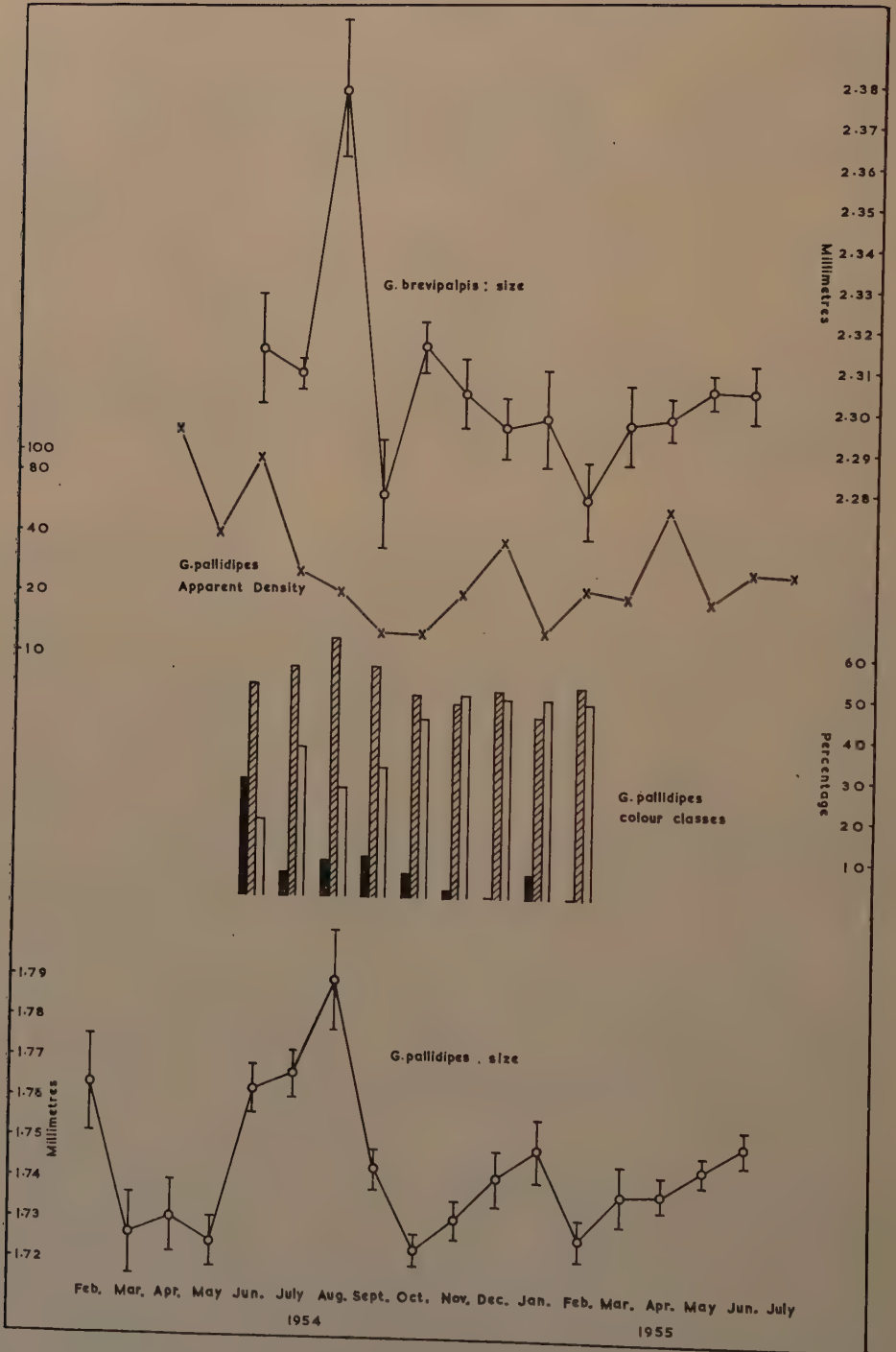


Fig. 3.—Variations in size, colour and apparent density of males of *G. pallidipes*, and in size of males of *G. brevipalpis*.

of catches". While it is interesting that the catches of *G. pallidipes* made in the way described resemble many other biological data in falling into a negative binomial distribution, nevertheless Table I is not an adequate summary of fig. 4. We saw that the pattern of fig. 4 fits in some degree the vegetation pattern

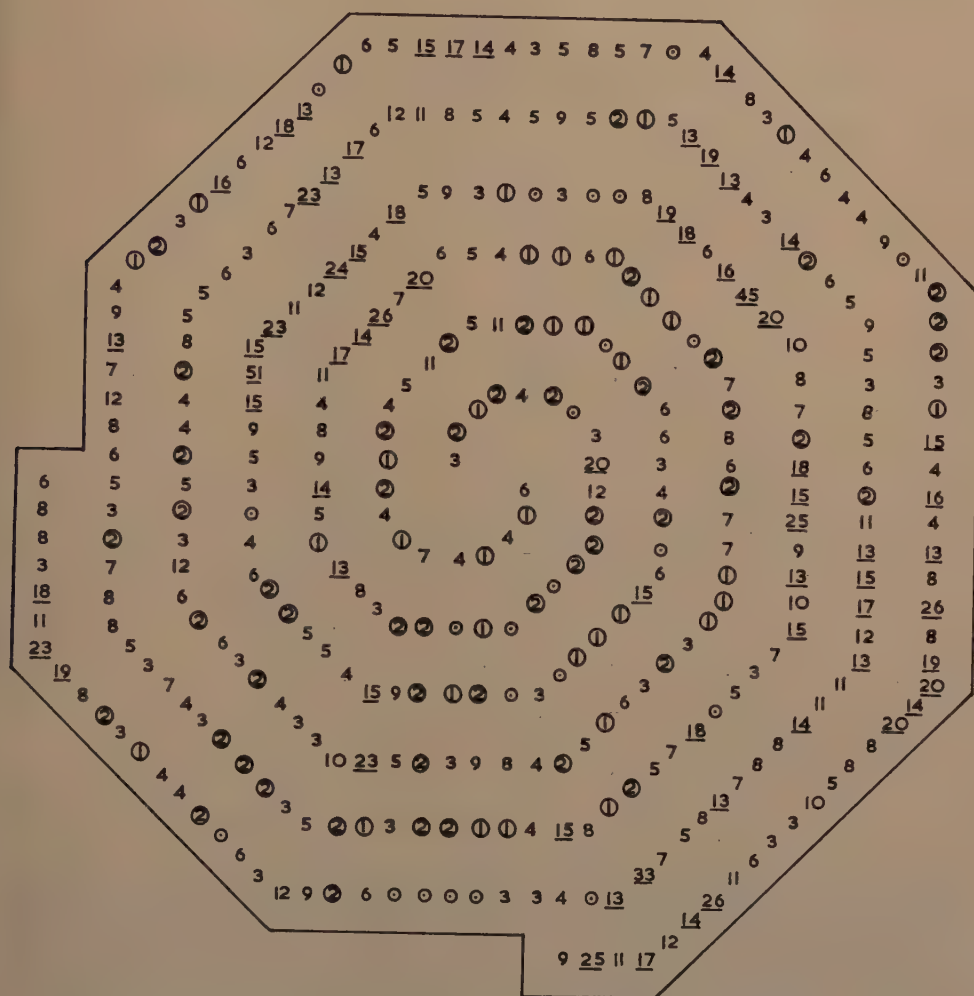


Fig. 4.—Total catches of non-teneral males of *G. pallidipes* at each catching post on the spirals, from January to July 1955. Catches of 13 and more underlined, of 2 and less ringed.

shown in fig. 1. The fit is perhaps not very striking, but whether illusory or not, it is by no means implicit in Table I. The figures on fig. 4 could be rearranged to indicate a quite different relation to the vegetation, or no relation at all, without affecting in any way the mathematical distribution as shown in Table I.

Day to day changes in the distribution of G. pallidipes.

When we examine the catch of *G. pallidipes* day by day, big changes are seen to occur in a short time. It is proposed to describe one very spectacular week in

detail, and then to consider to what extent it is representative of the whole period of the investigation. The distribution of catches on 18th June 1954 is shown in fig. 5, and is one which excites no special comment. The catch of 18 at one place (No. 147) was unusual, but catches of 17 had occurred twice before in that month

TABLE I.

Summary of the total catches of non-fertile males of *G. pallidipes* on the spiral, January-July 1955.

Number caught at a post	Observed	Expected
0	23	29.73
1	35	35.34
2	50	35.65
3	35	33.81
4	30	31.06
5	30	27.97
6	25	24.85
7	15	21.86
8	26	19.10
9	12	16.58
10	4	14.34
11	12	12.35
12	9	10.60
13	12	9.07
14	8	7.75
15	11	6.61
16	3	5.62
17	5	4.78
18	6	4.05
19	4	3.43
20	5	2.91
21	0	2.46
22	0	2.08
23	4	1.75
24	1	1.48
25	2	1.25
26	3	1.05
27+	3	5.50

(Table II). The distribution can be fitted to a negative binomial, but if fig. 5 were the only information available one could by no means predict the distribution shown in fig. 4, which, as stated above, is essentially similar to the total for April-December 1954. After 18th June, no more work was done until 22nd June. On that day, the tracks of a herd of elephants were noted in many places, and the distribution of *G. pallidipes* was very different from that on 18th June, both on the ground (fig. 6) and in terms of the negative binomial (Table II). On the ground, the distribution was characterised by a marked concentration in the vicinity of No. 332, and mathematically by unprecedentedly low values of k . On 23rd June, the record is incomplete, as the party which began work at No. 374 ran out of paint and abandoned work at No. 327; they had, however, already passed through the concentration at No. 332 and comparison of figs. 6, 7 and 8 suggests that the amount of information lost was not great. The day was characterised by the persistence of the concentration at No. 332 and by the appearance of a new concentration, quite unexpected on the basis of the previous days' results, about No. 118. On 24th June, further development of the concentration at No. 118 had occurred (fig. 8). On 25th June, although the concentration at No. 332

TABLE II.
Summary of catches of non-feneral males of *G. pallidipes* in June 1954, showing the number of posts at which different numbers of flies were taken.

Date	Number of flies																									Total	Mean	k
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25		
3.vi.54	304	26	12	3	2	1	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	89	.2550	.1229
4.vi.54	307	24	7	5	2	2	1	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	65	.2436	.1145
5.vi.54	313	22	8	2	3	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	84	.1834	.1127
8.vi.54	312	20	9	4	1	2	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	71	.2092	.0982
9.vi.54	306	28	7	4	2	1	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	84	.2407	.1187
10.vi.54	307	21	7	12	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	80	.2292	.1201
11.vi.54	317	21	4	3	3	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	55	.1576	.1051 *
15.vi.54	304	26	14	3	1	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	76	.2178	.1621
16.vi.54	311	23	10	2	2	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	65	.1862	.1217
17.vi.54	319	21	6	-	-	-	1	1	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	56	.1605	.0828 *
18.vi.54	297	30	10	7	2	1	-	1	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	109	.3123	.1341
22.vi.54	319	12	6	4	-	1	1	2	1	1	-	1	-	-	-	-	1	-	-	-	-	-	-	-	-	105	.3009	.0434
24.vi.54	295	20	13	2	4	1	1	2	1	-	-	-	-	1	2	-	-	2	-	1	1	1	-	-	-	283	.8109	.0645
25.vi.54	299	15	9	7	8	2	-	3	-	2	-	1	-	1	1	-	-	1	-	-	-	-	-	-	1	212	.6075	.0669
28.vi.54	307	23	8	6	1	-	2	1	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	90	.2579	.0972
29.vi.54	306	24	13	4	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	70	.2006	.1659
30.vi.54	322	18	5	2	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	42	.1203	.1070 *

* Maximum likelihood values: other k values from equation (5) of Bliss (1953). The two right-hand columns give the parameters defining negative binomial distributions for each day. The expected values calculated from these values agree with the observed values on every day, P varying from .051 to .95. The standard error of k on 22.vi.54 is .01065, giving fiducial limits .0221 and .0647.

was still recognisable, the one at No. 118 was bigger (fig. 9). Finally, after the week-end, on 28th June, the concentrations at Nos. 332 and 118 had both disappeared (fig. 10).

It is tempting to suppose that the visit of the elephants on 21st June was responsible for the remarkable events summarised in the last paragraph. Certainly one can understand that such incidents could give rise to the "story so persistently urged" about the mass movement of tsetse communities with large mammals. There are, however, difficulties in the way of accepting that the elephants were causal. In the first place we know of no mechanism by which such dense and small concentrations might be formed. We know that male flies (it is to be remembered that these observations are confined to this sex, females being at all times excessively rare in the catches) follow moving objects, and that large objects are more attractive than small ones. We may concede the probability that the smell of elephants is attractive, and so imagine a herd acquiring a large number of attendant flies. It is not easy to understand such a swarm all leaving the elephants in an area less than 200 yd. long and less than 150 yd. wide. A further difficulty is that whereas the concentration near No. 332 was first seen on 22nd June, that near Nos. 118-280 was not apparent until 23rd June. The fact (Table III) that the flies at 118-280 were less hungry than those at 332 might suggest that those flies which fed left the elephants in one place while those which did not feed left them in another. This unconvincing hypothesis does nothing to explain the tendency, apparent in both concentrations (Table III), for hunger to decrease as time went on.

TABLE III.

Comparison of hunger of two concentrations, that near No. 332, and that near Nos. 118-280.

	No. 332			No. 118 & 280		
	Total	No. caught 'up'	Percentage 'up'	Total	No. caught 'up'	Percentage 'up'
22.vi.54	57	13	23	—	—	—
23.vi.54	169	23	14	25	2	8
24.vi.54	128	21	16	101	5	5
25.vi.54	44	2	5	98	3	3
Totals	398	59	15	224	10	4

The difference between the totals, 59/398 as against 10/224, is highly significant ($\chi^2=15.6$, $P<.001$).

However, the elephants could still have caused the concentrations although we do not know how. As the work went on for 16 months, one might hope to be able to establish whether an association exists between elephants and concentrations of tsetse. Before attempting this, however, a number of difficulties have to be appreciated.

It so happened that concentrations as obvious as those shown in figs. 6-9 did not appear again. Examination of the catches from day to day usually revealed conditions resembling those in figs. 5 and 10, with the majority of the catch in a limited portion of the area, but this limited fruitful portion constantly changed position, in an irregular and unpredictable manner, and the vague definitive pattern (fig. 4) became apparent only when a large number of days were totalled. Sometimes, however, the fruitful portion was very small, and 22nd June falls into place as a day when the fruitful portion was very small indeed. It became evident that

TABLE IV.

The daily total catch of non-teneral males, together with the size and position of A-50 (the minimum polygon without re-entrant angles enclosing 50% of the catch).

			A-50			
			Catch (no. of flies)	Actual no. of posts	Percentage	Sectors involved
6.iv.54	167	111	32	VIII, I, II
8.iv.54	199	144	41	V, VI, VII, VIII
9.iv.54	116	48	14	VI, VII
10.iv.54	142	78	22	VI, VII, VIII
12.iv.54	120	74	21	VI, VII, VIII
13.iv.54	122	55	16	I, II, III
14.iv.54	135	64	18	I, II, III
15.iv.54	85	57	16	I, II, III
21.iv.54	142	80	23	I, II, III
22.iv.54	238	121	35	VI, VII, VIII
23.iv.54	326	168	48	IV, V, VI, VII, VIII
26.iv.54	67	42	12	VIII, I
27.iv.54	31	83	24	VIII, I, II
28.iv.54	60	64	18	II, III, IV
3.v.54	60	17	5	II, III
5.v.54	23	30	9	II, III
6.v.54	42	45	13	I, II, III
7.v.54	30	13	4	II
10.v.54	70	67	19	VI, VII, VIII
12.v.54	47	101	29	V, VI, VII
14.v.54	6	8	2	III, IV
17.v.54	44	74	21	I, II, III
18.v.54	37	105	30	V, VI, VII
19.v.54	13	60	17	II, III, IV, V, VI
20.v.54	34	47	13	VI, VII
24.v.54	61	47	13	VIII, I
25.v.54	55	32	9	VIII, I
26.v.54	53	25	7	VI
27.v.54	60	43	12	VI, VII
31.v.54	58	174	50	III, IV, V, VI, VII
3.vi.54	89	64	18	II, III, IV
4.vi.54	85	31	9	I, II
5.vi.54	64	39	11	I, II, III
8.vi.54	71	71	20	VIII, I, II
9.vi.54	84	31	9	VIII, I, II, III, IV
10.vi.54	80	86	25	VIII, I, II, III
11.vi.54	55	37	11	VIII, I, II

TABLE IV—continued.

	A-50			
	Catch (no. of flies)	Actual no. of posts	Percentage	Sectors involved
15.vi.54	76	47	13	I, II, III
16.vi.54	65	81	23	VII, VIII, I
17.vi.54	56	48	14	VIII, I, II
18.vi.54	109	39	11	VIII, I
22.vi.54	105	6	2	II, III
23.vi.54	249	4	1	II, III
24.vi.54	283	35	10	II, III
25.vi.54	212	24	7	V, VI
28.vi.54	90	76	23	V, VI, VII
29.vi.54	70	108	31	VIII, I, II, III
30.vi.54	42	95	27	VIII, I, II, III
2.vii.54	44	47	13	VIII, I
5.vii.54	20	23	7	I, II, III
6.vii.54	18	69	20	VIII, I, II
7.vii.54	24	84	23	I, II, III
8.vii.54	36	23	7	VIII, I, II
14.vii.54	7	22	6	III, IV, V,
15.vii.54	21	28	8	I, II, III, IV, V, VI
16.vii.54	25	47	13	I, II, III
17.vii.54	12	15	4	VIII, I
20.vii.54	20	27	8	II, III, IV
21.vii.54	22	44	13	I, II, III
22.vii.54	44	80	23	IV, V, VI
23.vii.54	34	75	22	V, VI, VII
26.vii.54	36	108	31	VIII, I, II
27.vii.54	31	39	11	VII, VIII, I
28.vii.54	44	18	5	VIII, I
29.vii.54	57	78	22	VIII, I, II, III

Roman numerals refer to the 8 sectors of the octagonal spirals, see fig. 1. The size of A-50 is defined by the number of catching posts it enclosed; at this period a full day's work comprised 349 posts.

an objective criterion of a concentration was required, and this desideratum has not been satisfactorily achieved. The parameter k of the negative binomial is not adequate. This is not only because, as already explained, many different arrangements on the ground can give exactly the same binomial distribution, but also because the negative binomial, being a description of the whole distribution, gives more weight (*e.g.*, on 24th June) to the 295 empty units than to two units of 25 which between them comprise more than one-sixth of the catch. The method already mentioned for defining catches above and below high and low thresholds (Ford & others, 1959) was in fact developed for dealing with these data, but it has not proved satisfactory for dealing with this particular problem. It provides an objective definition of a high catch at any particular catching post, but it fails to define satisfactorily a group of such high catches. For example, on 22nd June (fig. 6) the mean catch was 0.3, so that catches of 2 and more are considered high. The concentration about No. 332 consists of 7 contiguous stations all with catches exceeding 2, and this may be accepted unquestionably as a concentration with the

TABLE V.

Density of concentration and total catch of flies in relation to presence of large game animals.

Date	A-50 (%)	Total catch	Game observations	Association
20.iv.54	—	—	Work interrupted : elephants & buffalo	
21.iv.54	23	142		
22.iv.54	35	238		—
23.iv.54	48	326		
3.v.54	5	60	No work since 28.iv.54	
6.v.54	13	42	Buffalo herd	
7.v.54	4	30		+
15.vi.54	13	76	Buffalo herd	
16.vi.54	23	65		—
17.vi.54	14	56	Buffalo herd	
18.vi.54	11	109		—
22.vi.54	2	105	Elephant herd	+
23.vi.54	2	249		
14.vii.54	—	7	Elephant herd	?
20.vii.54	8	20	Elephant herd	
21.vii.54	13	22		—
28.vii.54	5	44	No large animals	—
4.viii.54	1	26	No large animals	—
5.viii.54	9	31	Buffalo herd	—
6.viii.54	14	17		—
11.viii.54	3	25	No large animals	—
7.ix.54	4	21	Elephant herd	+
13.ix.54	—	16	Elephant herd	?
20.ix.54	—	8	Elephant herd	?
7.x.54	—	7	Elephant herd	?
10.xi.54	1	44	Buffalo herd on 8th	+
15.xi.54	4	34	No work since 12th	
23.xi.54	15	29	Buffalo herd	—
16.xii.54	4	40	Buffalo herd	+
3.i.55	12	50	Buffalo herd	—
25.i.55	—	2	Buffalo herd	?
2.ii.55	1	20	Buffalo herd	
3.ii.55	5	68	Buffalo herd	+
4.ii.55	1	13		

TABLE V—*continued*.

Date	A-50 (%)	Total catch	Game observations	Association
15.ii.55	2	33	Elephant herd	+
25.ii.55	11	40	Elephant herd 23rd	—
2.iii.55	—	7	Buffalo herd	?
22.iv.55	10	32	Elephant herd	—
25.iv.55	3	122	Elephant herd	+
7.v.55	1	28	Elephant herd 5th	+
23.v.55	2	45	No work since 10th	
26.v.55	—	7	Elephant herd	?
3.vi.55	8	33	Elephant herd on 2nd	—
20.vi.55	5	25	No large animals	—

Summary of Table V.

	Total occasions	Positive	Negative	Doubtful	No evidence
Concentrations (A-50=1-5) ..	16	9	4	—	3
Elephants	15	5	5	5	—
Buffalo	12	4	6	2	—

possible addition of the catch of 2 at No. 90. But at the same time there is a catch of 7 at No. 311, and not a great way off are several catches of 3 and 2. A little reflection will show several ways of grouping these high-yielding stations into one or more concentrations. No objective way has been found of choosing between several possibilities in such cases.

Finally, since the essence of a concentration is many flies in a small space, it seemed that a satisfactory definition might be found in terms of the area required to contain a certain fraction of the total catch. By analogy with the widely used LD50, 50 per cent. of the total catch was adopted as the standard, and on figs. 5-10 have been drawn polygons enclosing the smallest possible area (without re-entrant angles) containing 50 per cent. of the total catch. This may be called A-50 (for area containing 50 per cent.). On this analysis, the 'normal' days 18th and 28th June each required more than 10 per cent. of the area to contain half the catch, while 22nd-23rd June appear as days of intense concentration with half the catch in less than 2 per cent. of the area. That this A-50 criterion is imperfect is admitted: it is proposed to use it until a better one is invented.

The A-50 values for each day of the first four months' work (April-July 1954) are given in Table IV together with the location of the A-50 polygons in terms of the eight sectors of the spiral (see fig. 1). It illustrates the variability of the data.

Before proceeding to the examination of the remainder of the data, some other difficulties must be mentioned. Elephants are dangerous animals, and standing

orders were to abandon work and come home as soon as they were sighted. Thus our records were necessarily incomplete on the days when they might have been most valuable. The anecdote of Morris (1960), who found the extraordinary catch of 283 flies in the battered cage of a trap which had been trampled by elephants, suggests what we may have missed. A second difficulty is apparent from inspection of fig. 6. The concentration about No. 332 was only just inside our study area; it might by chance have been just outside, in which case the catch of 22nd June would have been only 47 instead of 105. Equally it can be seen that the concentration was on the edge of the area trampled by the elephants; clearly the elephants might pass close by the study area and deposit a concentration just inside it and leave few or no tracks in the study area. Thus, even if the elephants caused and never failed to cause concentrations, one could not expect to establish by observations of this type either that (a), whenever elephants appeared on the spiral, so did concentrations of tsetse, or (b), whenever concentrations appeared on the spiral, elephants had recently passed. One would, however, expect coincidence to be normal: numerous instances of elephants without concentrations, or concentrations without elephants, would suggest that their relationship is not causal.

The relevant observations are summarised in Table V, adopting the arbitrary A-50 figure of 5 per cent. or less as indicating a concentration of *G. pallidipes*. Concentrations, by this criterion, appeared on 16 occasions (counting times when a concentration persisted for more than one day, as on 22nd–23rd June, as one occasion). On four occasions there were no large animals recorded, on three occasions they occurred after a break of several days in the work, but on nine occasions they occurred within a day or two of the passage of herds of elephants or buffalo. Elephant herds passed on 15 occasions; on five of these, concentrations of *G. pallidipes* occurred, on five they did not, and on five the catches of *G. pallidipes* fell very nearly to zero. This is perhaps in accordance with the proposition that elephants cause concentrations; although Morris (1960) seems to think that there are always more flies near big game, it would seem that a concentration can only be formed by taking flies out of a large space and putting them in a small one. Buffalo herds passed on 12 occasions; on four of these, concentrations of *G. pallidipes* occurred, on six they did not, and on two the catches of *G. pallidipes* fell very nearly to zero.

In compiling Table V, the many occasions on which one or two elephant or buffalo were recorded have not been included. If such occasions were included the number of occurrences, and of negatives, would be greatly increased, but it is herds with which we are concerned. On the other hand, on the four occasions on which concentrations of *G. pallidipes* occurred in the absence of large animals, these were completely absent, not even the tracks of solitary individuals being seen.

As noted above, if elephant and buffalo herds caused and always caused concentrations of *G. pallidipes*, one could not, in an area of this size, expect to see them always together. One could not, in fact, expect more coincidence than appears in Table V. On the other hand, is Table V compatible with the null hypothesis, that concentrations are either (a) imaginary, or (b) caused by something other than big game? Most people, I think, will accept that a 50 per cent. catch in less than 5 per cent. of the area does represent a concentration. If this be conceded, it would seem improbable, though not impossible, that the position shown in the summary of Table V could arise unless the concentrations were in some way the result of the presence of the large animals. Somewhat hesitantly, then, I conclude that elephants and buffaloes probably cause concentrations of *G. pallidipes*. This is also the view of Morris (1960); it is of interest that the extraordinary catch, mentioned above, of 283 flies in one trap contained the usual (for traps) high percentage of females, suggesting that the concentrations do not consist merely of males, as might appear from the present work.

Apart from the very marked concentrations with A-50 less than 5 per cent., the observation that such conditions as those shown in figs. 5 and 10 are usual is illuminating. It seems that vague and ill-defined groups of flies are constantly wandering about in the bush, now spreading out, now coalescing with other groups, and from time to time responding to a powerful stimulus such as a herd of elephants. Patterns such as those shown in fig. 4, customarily referred to as "relation to the vegetation" have meaning only in relation to the sum of many days' observations. If this is true of *G. pallidipes* in other places, and of other species of *Glossina*, it explains the very marked day-to-day variations in the catch on fixed routes, whether made by fly-rounds (Ford & others, 1959; Glasgow, 1961) or by lines of traps (Glasgow & Duffy, 1961). Such variations are now seen to be due, not to some mysterious fault in the techniques employed, but to an inescapable property of the populations being studied.

Summary.

The daily distribution of *G. pallidipes* Aust. was studied on a spiral fly-round covering 180 acres in the South Busoga forest of Uganda in 1954-55. *G. brevipalpis* Newst. also occurred in the forest, and, rarely, *G. palpalis fuscipes* Newst. Seasonal changes occur in the size of *G. pallidipes*, but are less marked than those reported from other places. The size of *G. brevipalpis* also varies with season. Seasonal colour changes in *G. pallidipes* indicate that in the driest season about half the puparia experience relative humidity as low as 70 per cent.

Catching was done at 349 (later increased to 373) points on the spiral. Mathematically, the catches on a single day, or the sum of many days, conformed to a negative binomial distribution. Although this indicates an uneven distribution, it is pointed out that one negative binomial is compatible with many different distributions on the ground. For this reason the negative binomial is not a satisfactory summary of data which at present can only be shown satisfactorily on a map.

Comparison of maps of catches on successive days shows a confusing pattern of vague and ill-defined patches of flies moving about in an unpredictable fashion. A definitive pattern appears only when many days' catches are summed. Such apparently random movement explains the high variance between days' catches previously reported. Sometimes very large numbers of *G. pallidipes* were found in a small area. As an index of such concentrations, the smallest area which could contain half the day's total catch was determined. Arbitrarily, when half the day's total catch was found in 5 per cent. or less of the total area, a concentration was said to exist. During 16 months' work, concentrations of tsetse, as defined by this criterion, were observed 16 times. Sometimes, but not always, the concentrations followed immediately after the passage of a herd of elephants or buffaloes, and it is concluded that these animals probably caused these concentrations, although the manner in which they may do so remains obscure.

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THE HISTOCHEMISTRY OF THE CHOLINESTERASES IN THE
CENTRAL NERVOUS SYSTEM OF SUSCEPTIBLE AND RESISTANT
STRAINS OF THE HOUSE-FLY, *MUSCA DOMESTICA* L.,
IN RELATION TO DIAZINON POISONING.

By FRANCES M. MOLLOY

Department of Insecticides and Fungicides, Rothamsted Experimental
Station, Harpenden, Herts.

(PLATES VII & VIII.)

The abundance of cholinesterase (ChE) acetylcholine (ACh), and choline acetylase (ChA) in insect nervous tissue has been well established (Chefurka & Smallman, 1955; Lewis & Fowler, 1956; and Colhoun, 1959*a, b*), but the exact rôles played by these substances in the insect have not yet been fully elucidated. There is no evidence of cholinesterase at the motor nerve endings in *Rhodnius prolixus* Stål (Wigglesworth, 1958), and Colhoun (1959*b*) was unable to demonstrate the presence of ACh, ChE and ChA in denervated roach muscle. Cholinesterase has, however, recently been demonstrated histochemically in the muscle receptor organs, and, more tentatively, in the motor nerve endings, of a crustacean, *Homarus americana* H.M. Edwards (Decapoda), by Maynard & Maynard (1960). This appears to be the first histochemical demonstration of cholinesterase at nerve endings among the arthropods. Cholinesterase seems to be confined to the nervous system in insects (Wigglesworth, 1959).

The insecticidal action of organophosphates is generally thought to be due almost entirely to their anticholinesterase action (Metcalf & March, 1949; Spencer & O'Brien, 1957; Smallman & Fisher, 1958), although Lord & Potter (1951, 1954), Potter & others (1957), Hopf (1952, 1954) and van Asperen (1958*a, b* and 1960*b*) stressed the possibility that inhibition of other esterases might also be involved, even if they are not necessarily the cause of death.

Biochemical studies of the *in vivo* action of organophosphorus insecticides are hampered by the mixing of tissues during their preparation for enzyme assay, and unduly high inhibition may occur in homogenates of whole insects as a result of poison which *in vivo* is far removed from the site of action. This may be avoided in part by removing the 'ineffective' inhibitor by such procedures as freeze-drying and chloroform extraction (Scaife & Shuster, 1960), or by protecting the enzyme by the addition of substrate (van Asperen, 1958*a*, 1960*a*). Both techniques give results which indicate that *in vivo* cholinesterase inhibition is not nearly so generally complete as was postulated by Smallman & Fisher (1958). The problem then arises as to whether such inhibition as does occur is highly localised and complete, in which case death might occur by a localised area ceasing to function, or whether the inhibition is generalised and only partial throughout the nervous system. This problem can be elucidated by the use of histochemical techniques.

The histochemical information on the effect of organophosphate poisoning on the enzymes of the insect nervous system is very sparse, in contrast to the wealth of biochemical information on this subject. Winton, Metcalf & Fukuto (1958) are the only authors who have studied the effect of *in vivo* inhibition by organophosphorus insecticides on the histochemistry of the insect nervous system. They found evidence of the inhibition of acetylcholinesterase in the cut ends of the nerve

cord of *Periplaneta americana* (L.) after poisoning with paraoxon, tetraethyl pyrophosphate (TEPP), or the oxygen analogue of Thimet sulphoxide. A partial staining reaction was produced with m-tert.-butylphenyl N-methylcarbamate, which, the authors implied, showed the reversibility of carbamate inhibition.

Wigglesworth (1958) described the normal distribution of non-specific esterases in the general body tissues, and of cholinesterase in the nervous system of *P. prolixus*, using histochemical methods. Iyatomi & Kanehisa (1958) studied the distribution of cholinesterase throughout the body tissues of unpoisoned *P. americana* with the histochemical technique of Koelle & Friedenwald (1949), without, however, determining whether the activity they found in certain organs of the body was due to the nervous elements in the tissues, or to activity of the tissues themselves.

It seemed useful to examine the distribution and localisation of cholinesterases in the nervous system of a different insect, and the extent to which these enzymes were inhibited by organophosphorus compounds.

The house-fly, *Musca domestica* L., known to be rich in cholinesterase, was chosen as the test insect because of the availability of flies bred under controlled conditions, and because an organophosphorus-resistant strain was available in addition to the normal, susceptible strain. It was hoped that a comparison of the effects of the poison on the enzymes in the nervous system of the two strains might be helpful in the understanding of the toxic effects of the poison.

So far, only the two large compound ganglia of the central nervous system have been studied.

The central nervous system of *M. domestica*.

A typical insect nerve ganglion contains a central neuropile or synaptic region consisting of densely packed nerve fibres and their branches from motor, sensory and association neurones. Surrounding the neuropile is a less densely packed layer of neurones and non-nervous tissue including neurosecretory cells and glial cells of four different kinds (Wigglesworth, 1959). Some of the glial cells form the myelin sheaths surrounding the motor nerve axons, and others form the perineurium (p.n., fig. 3), or sheath cells (Ashhurst, 1959) which lie just inside the protective connective tissue sheath or neurilemma (n.l., fig. 3) surrounding the ganglion. The perineurium is concerned with active ionic regulation between the nervous system and the blood.

In a primitive insect there is a dorsal cephalic ganglion or brain which is connected by a pair of circumoesophageal connectives to a ventral chain of ganglia, which are primitively segmental in arrangement. The different orders of insects show varying degrees of specialisation from this primitive arrangement, and this specialisation and concentration is greatest in the higher Diptera in which there are only two ganglionic masses connected by a ventral nerve cord, the double nature of which is only seen in sections. The central nervous system of *M. domestica* is shown in fig. 1.

In *Musca*, the supra- and suboesophageal ganglia which together form the brain are almost completely fused together, with just a small hole (oes. f.) remaining between the two to serve as a passageway for the narrow oesophagus and its related structures (fig. 2).

The ventral thoracic ganglion (fig. 1) is a compound ganglion consisting of three pairs of thoracic ganglia and the fused abdominal ganglia. A diagrammatic horizontal section of the thoracic ganglion is shown in fig. 3. The neuropile of this ganglion retains its lobed structure corresponding with the individual ganglia from which it is derived.

The compound supraoesophageal ganglion (fig. 2) consists of three pairs of ganglia (Snodgrass, 1935): protocerebral (p.l.), deutocerebral (d.l.) and tritocerebral (t.l.). The large size of the lateral pair of optic lobes (o.l., fig. 1) which are part

of the first or protocerebral ganglia, is due to the specialisation of the large compound eyes. Each optic lobe contains three sensory and association synaptic regions, which are all concerned with sight. These are the outermost lamina ganglionaris (l.g.), adjacent to the base of the retinal cells (r.c.); the medulla externa (m.e.) and the medulla interna (m.i.). The three regions are connected by the outer and inner chiasmata (o.c. and i.c.), so called because of the crossing-over of nerve fibres within them. The optic centres of a related form, *Calliphora vomitoria* (L.), were described in great detail by Cajal & Sánchez (1915) and Cajal (1918).

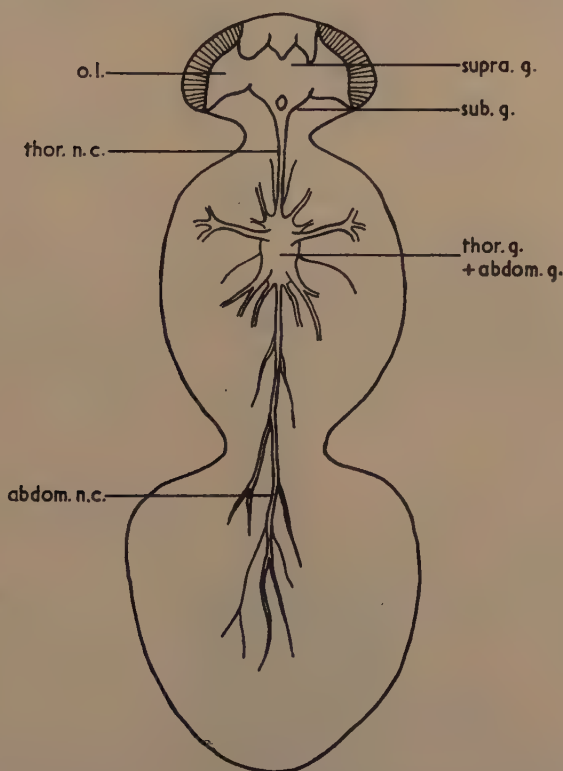


Fig. 1.—The central nervous system of *M. domestica* (dorsal view). abdom. n.c., abdominal nerve cord; abdom. g., abdominal ganglion; o.l., optic lobe; sub. g., suboesophageal ganglion; supra. g., supraoesophageal ganglion; thor. n.c., thoracic nerve cord; thor. g., thoracic ganglion.

The central region of the supraoesophageal ganglion is the main co-ordination centre for association fibres connecting the motor and sensory systems. The deuto- and tritocerebral lobes contain the motor and sensory centres for control of the antennal and mouthpart segments. Pringle (1940) showed electrophysiologically that the thoracic ganglia of *P. americana* are responsible for reflex locomotory activity. However, excitation and maintenance of locomotory activity is probably regulated by the suboesophageal ganglion which, in its turn, is controlled by inhibitory impulses from parts of the supraoesophageal ganglion (Roeder, 1953).

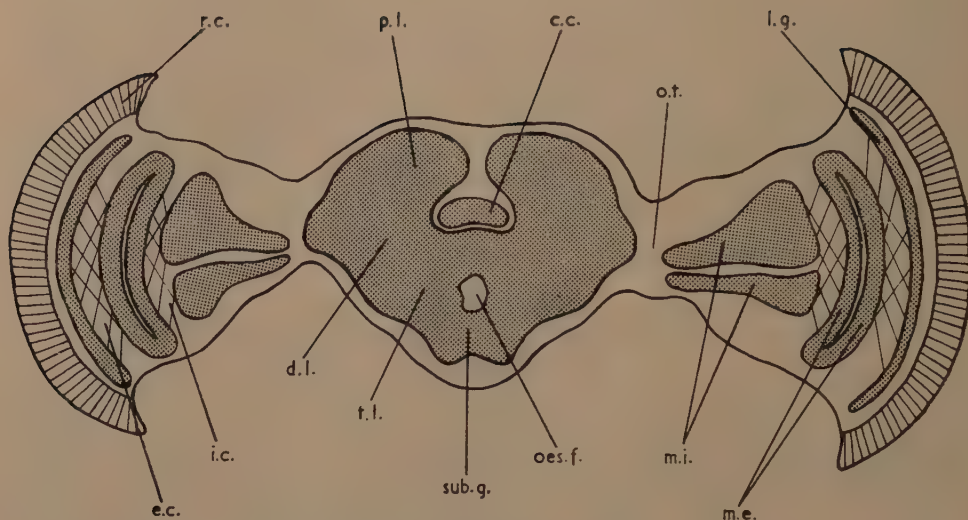


Fig. 2.—Semi-diagrammatic transverse section of the brain of *M. domestica*. Synaptic regions (neuropile) stippled; cell-body region unstippled. c.c., corpus centrale; d.l., deuto-cerebral lobe; e.c., external chiasma; i.c., internal chiasma; l.g., lamina ganglionaris; m.e., medulla externa; m.i., medulla interna; oes.f., oesophageal foramen; o.t., optic tract; p.l., protocerebral lobe; r.c., retinal cells; sub.g., suboesophageal ganglion; t.l., tritocerebral lobe.

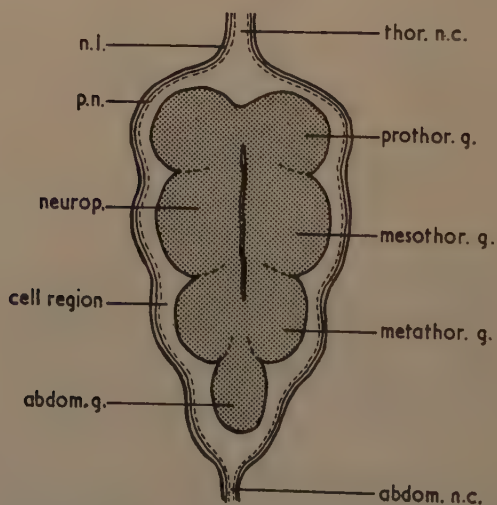


Fig. 3.—Semi-diagrammatic horizontal section of the compound thoracic ganglion of *M. domestica*. Synaptic areas (neuropile) stippled; cell-body region unstippled. abdom. g., abdominal ganglionic mass; abdom. n.c., abdominal nerve cord; mesothor. g., mesothoracic ganglion; metathor. g., metathoracic ganglion; neurop., neuropile; n.l., neurilemma (connective tissue sheath); p.n., perineurium (sheath cells); prothor. g., prothoracic ganglion; thor. n.c., thoracic nerve cord.

Material and methods.

Rearing and toxicological methods.

The test insects were a normal susceptible strain of *M. domestica* reared at 26°C. and 50 per cent. R.H., and a diazinon-resistant strain, SKA, reared under continuous selection pressure of approximately 25 per cent. The adults of each generation of resistant flies were exposed to strips of filter paper impregnated with diazinon to kill about 25 per cent. of the flies, and rearing was continued with survivors. The SKA strain was originally obtained by crossing two strains, one from Sacca of Italy and the other from Keiding of Denmark.

Only 3- to 4-day-old female flies of either strain were used for the experiments.

Application of poison.

The poison was administered by topical application after immobilisation of the flies by cooling. Female flies were sorted into petri dishes (15 per dish) in a cold cabinet (5–10°C.) and allowed to revive. They were fed with milk paste and 10 per cent. sucrose absorbed in cotton-wool rolls. On the same or the following day, the flies were again cooled and treated with a drop (1 μ l.) of diazinon in acetone on the ventral part of the thorax (unless otherwise stated) between the bases of the legs. The diazinon (O,O-diethyl O-2-isopropyl-4-methyl-6-pyrimidinyl phosphorothioate) was either technical grade (85%) or pure active ingredient.

Batches of approximately 90 flies were treated at each concentration used. After treatment, about 60 flies were kept at 25°C. in petri dishes containing food in order to assess the percentage kill after 24 hours. The remaining 30 flies were placed individually in small tubes plugged with cotton-wool moistened with 10 per cent. sucrose and also kept at 25°C. These flies were examined at various times after treatment, when samples of living, dead and affected flies were placed in the deep-freeze at –16°C. to await histological examination.

Topical application on flies held by suction.

This alternative method was developed by Sawicki (1961) working in this department. The flies were held by the suction of a vacuum cleaner against a Terylene gauze pad, where they were sorted and treated rapidly without cooling or the use of anaesthetics. After treatment, the flies were released into glass tubes covered with Terylene gauze. A drop size of 0.125 μ l. or 0.25 μ l. was used with this apparatus to avoid loss of poison in the air stream which occurs when larger drops are used.

Classification of flies after poisoning with diazinon.

Flies examined at intervals after poisoning with diazinon may be classified in five stages, which more or less correspond with those outlined by Tattersfield & Potter (1943) for *Tribolium castaneum* (Hbst.) after pyrethrum poisoning:—
1. *Unaffected*. Normal activity; 2. *Slightly affected*. Behaviour nearly normal, but with dragging of one or two legs; drooping of wings; 3. *Badly affected*. Hyperactivity; rapid spinning movements in flight or on a surface; loss of balance and directional movements; side effects such as extrusion of ovipositor; continual cleaning movements; 4. *Moribund*. Prostrate on back and unable to regain normal posture; occasional twitching or rapid movement of legs; 5. *Dead*. Motionless for some time.

It is very difficult to distinguish between stages 4 and 5, since the flies may be completely moribund and apparently dead for some time, and yet when stimulated, wave their legs in the air either feebly or fairly vigorously. A fly that has reached stage 3, i.e., is badly affected, within three or four hours of poisoning does not recover completely, and is usually dead 24 hours after poisoning. Thus, there is no marked knockdown followed by complete recovery of the flies as in pyrethrum poisoning, and in flies poisoned with TEPP (Stegwee, 1960).

Histochemical methods.

Flies taken from the deep-freeze were dissected under water. The brains and thoracic ganglia were dissected out and placed in Ringer solution (Hoyle, 1953) after removal of as much tracheolar and glandular tissue as possible. It was found by experiment that no *in vitro* inhibition of the cholinesterase in the central nervous system occurred if the thoracic ganglia were immersed for up to 30 minutes in a 2×10^{-6} per cent. solution of diazinon in water. This is a much higher exposure time and concentration than could arise under these conditions of dissection, if there was any contamination of the dissecting water by residual diazinon on the surface of the fly.

The ganglia were then fixed in absolute acetone at -16°C . for one or two hours, which helped penetration of the substrate during the subsequent incubation. Excess acetone was drained off with filter paper and the ganglia were then transferred to gum-sucrose (Holt & Withers, 1958) at 2°C . for 1-6 hours for frozen sectioning, or into sodium sulphate (sat. solution) to be incubated whole. During transference, the vessels containing the solutions were held in a cooling bath at -10°C . to prevent denaturation and inhibition of the enzyme by acetone. Frozen sections of the brains, $25-30\mu$ thick, were cut in frozen gum-sucrose solution which supported the tissue whilst it was being cut. Frozen gum-sucrose is softer and does not chip like pure ice. The frozen sections were removed from the knife with a brush or were allowed to fall directly into a dish of sodium sulphate. The thoracic ganglia were usually incubated whole.

Cholinesterases were determined by the thiocholine method of Koelle & Friedenwald (1949), as modified and simplified by Gomori (1952). The substrates were acetylthiocholine iodide or butyrylthiocholine iodide, which are more specific for cholinesterases than other histochemical substrates.

The chemistry of this method consists essentially of two stages (Malmgren & Sylvén, 1955): (1) Incubation in a substrate medium containing Cu^{++} ions and a thiocholine ester. The ester is hydrolysed by the enzyme in the tissue, and the thiocholine liberated is precipitated as copper thiocholine sulphate. (2) The colourless copper thiocholine is converted by treatment with ammonium sulphide to copper sulphide, which is brownish black.

Twenty mg. of substrate ester was added immediately before use to 10 ml. of stock solution made up as follows: CuSO_4 0.3 g.; glycine 0.375 g.; MgCl_2 1.0 g.; maleic acid 1.75 g.; N (4%) NaOH 30 ml.; 40 per cent. Na_2SO_4 170 ml. The final concentration of acetyl- or butyrylthiocholine was about $6 \times 10^{-3}\text{M}$. The sections were incubated at 25°C . and at pH 6.4. The incubation time was kept constant throughout each experiment. Twenty minutes were sufficient to give adequate staining in sections of control flies. The sections were removed from the incubation solution with a small spatula, and washed in two changes of saturated sodium sulphate. They were then placed in about 10 ml. dilute (approx. 5%) yellow ammonium sulphide solution, in which the sections containing active cholinesterase turned dark brown or black very rapidly. After development of the stain, the sections were washed in two changes of distilled water, dehydrated, cleared, and mounted in Canada balsam. To prevent fading, prolonged immersion in xylene, particularly in strong light, was avoided. To prevent curling up of the sections during xylene immersion they were sometimes transferred direct from alcohol on to clean, dry slides, blotted firmly with a hard grade of filter paper (Whatman 544), dehydrated, cleared in xylene and mounted *in situ*.

The precision of localisation of the enzyme by this method depends on the length of incubation at a given temperature, and on the pH. With a low pH (Gerebtzoff, 1959) there is a loss of sensitivity but a gain in localisation. With a longer time and a relatively high temperature, diffusion artifacts increase with a consequent blurring of the stain and loss of localisation.

A quantitative estimation of the enzyme activity after different treatments was

not attempted. A visual comparison of the density and localisation of staining in poisoned and unpoisoned flies was done on the histological rather than the cytological scale because of the necessity of examining as many individuals as possible after each treatment.

Experimental results.

Distribution of cholinesterase in the nervous system.

The central nervous system of *M. domestica* is very active in hydrolysing both acetylthiocholine and butyrylthiocholine when examined by Gomori's (1952) modification of the thiocholine technique, and the precise localisation of the enzymic activity is made difficult by the large amount present.

In an acetone-fixed frozen section of the brain of an untreated fly, the cholinesterase is distributed throughout the section and is not always confined to the neuropile or synaptic region (fig. 2 and Pl. VII, fig. 1), but extends to the peripheral neuronal region and the perineurium (cf. Wigglesworth, 1958). Occasionally the synaptic areas are more darkly stained than the surrounding cell areas in some relatively thin sections (20μ), but this is unusual.

Apart from an exceptionally high concentration of cholinesterase in the lamina ganglionaris (l.g., fig. 2), which is an association centre receiving post-retinal fibres, there are not usually any other very dark areas in untreated flies.

The compound thoracic ganglion, when incubated whole, stains very darkly (Pl. VIII, fig. 1), especially in the peripheral region surrounding the neuropile. The peripheral region contains the nerve cell bodies and non-conducting tissue including the perineurium, which is concerned in ionic regulation. The protective outer sheath or neurilemma shows no cholinesterase activity. The presence of cholinesterase in the outer layers of the ganglion, including the perineurium, and in the nerve roots arising from the thoracic ganglion, is characteristic of unpoisoned flies.

The centre of the thoracic ganglionic mass, when incubated whole, although obscured by the overlying dark perineurium, is often lighter in colour. This appearance is sometimes seen also in thick sections of the brain (100μ). Wigglesworth (1958) observed a similar reaction in the thoracic ganglion of *Rhodnius* when incubated whole with the same substrate. This effect seems to be due to a slowing down in the rate of penetration of the substrate into the interior of the ganglion since, when incubation is prolonged, the ganglion becomes very densely stained throughout. In horizontal sections of the ganglion cut at $25-40\mu$, the substrate is readily available to the central region and then the outer regions of the neuropile are comparatively more darkly stained than the perineurium surrounding it.

The fairly uniform distribution of cholinesterase, as shown by the stained region extending to the periphery of the brain and thoracic ganglion typical of living and unpoisoned flies, is also typical of flies which are dead from natural causes, even when they have been dead for several hours.

The distribution and amount of cholinesterase in unpoisoned flies is the same in both the normal susceptible strain and the diazinon-resistant strain.

A comparison of the two substrates shows that the distribution of hydrolysis of butyrylthiocholine is very similar to that of acetylthiocholine. There is the same general distribution throughout the central nervous system, but the rate of hydrolysis of butyrylthiocholine is slightly lower.

The effect of diazinon poisoning on the distribution of cholinesterase in a susceptible strain.

Diazinon was chosen for use in these experiments since a diazinon-resistant strain of *M. domestica* was available for comparison with the normal susceptible strain. Diazinon is converted in the insect body to its oxygen analogue (O,O-diethyl

O-2-isopropyl-4-methyl-6-pyrimidinyl phosphate) (Krueger, O'Brien & Dauterman, 1960), a more potent anticholinesterase.

The regions where cholinesterase is inhibited are shown by a lack of stain, or a reduction in intensity of the stain as compared with an unpoisoned control section incubated in the same way.

Susceptible strain

In general, the degree of inhibition of cholinesterase in the brain and thoracic ganglion of the house-fly increases with increasing doses of poison, and with time after poisoning. The extent of cholinesterase inhibition may be roughly correlated with the outward behaviour of the fly at any particular time after treatment. This correlation is not a strict one, however, and there is a good deal of variation between individual flies with the same poison symptoms.

The inhibition is progressive from the outer peripheral region of the ganglion inwards; the thoracic ganglionic mass is usually affected before the brain.

Flies outwardly unaffected by sub-lethal doses of diazinon (stage 1), *i.e.*, the surviving flies taken 24 hr. after an LD40-LD90 dose, do not usually show any inhibition of cholinesterase. When they do, it is very slight, and is confined to the nerve roots entering the thoracic ganglion.

A fly slightly or badly affected by diazinon poisoning first shows cholinesterase inhibition in the thoracic ganglion as a lightening or a complete lack of staining in the outermost region, including the perineurium and other non-conducting tissue, and probably in the neurones (white areas in fig. 3). The nerve roots of the larger nerves are also rapidly affected.

Similarly, the part of the brain which is first affected is the peripheral neuronal region (Pl. VII, fig. 2). The whole of the suboesophageal ganglion (sub. g., fig. 2), concerned with the control of locomotor activity, is often also rapidly affected (Pl. VII, fig. 3); this may account for the changes in locomotory behaviour of poisoned flies. Other rapidly inhibited areas in the brain are the lamina ganglionaris (*l.g.*, fig. 2), which is a purely sensory area concerned with visual stimuli, and parts of the deutocerebral and tritocerebral lobes, which control the antennal and mouth-part segments (*d.l.* and *t.l.*, fig. 2 and Pl. VII, fig. 3).

The extent of inhibition is of course greater in flies which are badly affected, moribund or dead (stages 3-5), especially after higher doses (*e.g.*, >LD70). The area where the cholinesterase is inhibited gradually encroaches on the neuropile in both the brain and the thoracic ganglion (Pl. VIII, figs. 2 & 3). Sometimes a narrow, darkly-staining region is visible around the uninhibited area of the neuropile, separating it from the inhibited zone. Such a dark border is not seen in untreated flies. In the thoracic ganglion, this stage of inhibition is typified by the 'mulberry' look of the ganglion, indicating its composite nature by outlining the edges of the neuropile of each of the constituent ganglia by a darker region of staining (Pl. VIII, fig. 2). In the brain, this stage is shown by the isolation of certain stained areas in the protocerebral lobes and medulla interna and externa of the optic lobes (*m.i.*, *m.e.*, fig. 2, and Pl. VII, fig. 3). These areas are further reduced in size a longer time after poisoning, but are often still quite darkly stained, indicating considerable enzyme activity, when the surrounding areas are completely colourless and unreactive. However, the degree of inhibition does not always extend further than the peripheral region of the central nervous system even in prostrate individuals, especially after lower doses of poison.

Thus, at the time of prostration, inhibition of cholinesterase in the central nervous system is rarely total; almost invariably there are certain areas showing high enzyme activity and other areas, mainly peripheral, showing complete or partial cholinesterase inhibition. Inhibition in the peripheral nervous system, other than the parts of the nerves immediately adjacent to the thoracic ganglion, has not yet been studied.

In certain rare instances, in flies taken 24 hr. after poisoning with a very high dose (over 99% kill), inhibition extends throughout the whole of the central nervous system until no enzymatically active areas are left, and the preparations are completely colourless.

It should be emphasised that usually even at the later stages of poisoning with the highest doses of poison used (over LD99), not all the cholinesterase is inhibited, and therefore total inhibition is not necessary to cause death. At prostration (generally 30 minutes to two hours after poisoning) a very small proportion of the total enzyme may be inhibited in affected flies, but the inhibition within the localised areas where it does occur seems to be complete.

In certain anomalous cases, flies examined at longer intervals after poisoning, and showing more advanced poison symptoms, had more active cholinesterase in the central nervous system than flies, given the same dose, but taken sooner after poisoning, when they seemed to be outwardly less affected. This suggested that some reactivation of the enzyme had occurred after its inhibition by the poison. However, it may be the result of using too small numbers of insects, since, inevitably, different flies must be examined at the different stages and individual variations are known to occur.

In order to investigate the immediate effect of poisoning with diazinon on ChE in the central nervous system, flies were treated as usual by topical application on the ventral part of the thorax. They were then decapitated one minute, five minutes, 10 minutes and 20 minutes after poisoning, in order to separate the brain from the site of application of the poison. The brain and thoracic ganglia were then immediately, and separately, dissected out under water, and fixed in acetone at -16°C . for one or two hours. The rest of the procedure was unchanged. No immediate inhibition was observed either in the brain or thoracic ganglion. The times after poisoning and before decapitation were extended to 40 minutes, one hour, two hours, three hours, 18 hours and 24 hours. Prostration occurred in most specimens between one and three hours, when small amounts of peripheral inhibition were evident in both the brain and thoracic ganglion. After this time, cholinesterase inhibition was progressive and did not show any distinct reactivation.

Effects of size and position of application of drop of poison.—Preliminary experiments were done to see whether the position of application and the size of the drop of diazinon affected the pattern of cholinesterase inhibition in the central nervous system.

The spread of drops of different sizes when placed on different parts of the fly was studied by means of fluorescent dyes dissolved in acetone, and examined under ultraviolet light. A drop of size 1 μl . placed on the head was largely transferred to the underlying filter paper, and also spread on to the anterior part of the thorax. A drop of the same size when placed on any part of the thorax immediately spread right around the thorax and on to the legs, but did not reach either the head or the abdomen. Similarly a drop of size 1 μl . placed on the abdomen rapidly spread over almost the whole surface of the abdomen but failed to reach the thorax. The spread of 0.5 μl . was much less, and a drop of size 0.25 μl . or less failed to spread very far from the actual site of application.

There were indications that a 1- μl . drop applied to different parts of the body, excluding the head, was more toxic on the ventral part of the thorax than elsewhere. However, histochemical examination showed no significant differences in the extent of cholinesterase inhibition either in the brain or in the thoracic ganglion when doses giving similar percentage kills were applied to different parts of the body. In a further experiment, where a smaller drop of diazinon solution, 0.5 μl ., was placed on the head, considerable cholinesterase inhibition had occurred after 18 hours in both the brain and the thoracic ganglion. There was relatively less enzyme left in the brain than would be expected from a similar dose on the thorax.

These experiments show that, although there may be a certain degree of

'swamping' of the enzyme due to excess inhibitor at the site of application, this excess does not completely inhibit the adjacent enzyme; it only slightly raises the level of inhibition over that of the enzyme in the central nervous system in other parts of the body. This was confirmed in a later experiment in which even smaller drops of poison solution (0.25 μ l.) were applied to the head and ventral parts of the thorax, respectively.

Comparison of different grades of diazinon.—In some of the earlier experiments described above, 85 per cent. technical grade of diazinon was used and it was thought advisable to compare directly the effects of pure diazinon and this technical grade. Results showed that with an LD50 there were comparable areas and amounts of inhibition of cholinesterase in the thoracic ganglion and brain in both cases.

Comparison between the effect of diazinon on the cholinesterase of susceptible and diazinon-resistant strains of M. domestica.

Females of a normal, i.e., susceptible, and a diazinon-resistant strain (SKA) of *M. domestica* were topically treated with diazinon in order to see whether there was a marked difference in enzyme inhibition in the two strains.

In the first experiment, technical diazinon (85% active ingredient) was used; the flies were all examined 18 hours after treatment, and the brains were sectioned at 100 μ . Acetylthiocholine and butyrylthiocholine iodides were used as substrates. In the second experiment, pure diazinon (99% active ingredient) was used at the same concentrations as above; the flies were examined at shorter time intervals after treatment, and the brains were sectioned at 25 μ . Acetylthiocholine iodide was the only substrate used.

Both experiments showed the same general effects: when a discriminating dose, i.e., 1 μ l. of 0.04 per cent. diazinon, was applied to the thorax, it gave an 85–99 per cent. kill in the susceptible strain, and approximately 5 per cent. kill in the resistant strain.

In the susceptible strain, the individuals which were badly affected within 40 minutes of treatment with this dose showed marked inhibition of cholinesterase in the peripheral part of both ganglia, in the lamina ganglionaris, suboesophageal ganglia and the deuto- and tritocerebral lobes of the brain (Pl. VII, figs. 2 & 3). These areas are mainly motor centres (except for the lamina ganglionaris, which is a purely sensory centre); the suboesophageal ganglion and thoracic ganglion between them play a large part in the control of locomotion in the insect (Roeder, 1953).

From two to 24 hours after poisoning with this dose, the affected individuals showed amounts of inhibition varying from peripheral to almost complete inhibition in both the brain and thoracic ganglion.

With the same dose, 95 per cent. of the resistant flies remained alive, and were examined at intervals after treatment. No inhibition was seen in the brain or thoracic ganglion (Pl. VII, fig. 4 and Pl. VIII, fig. 4), and only a very small amount in the nerves arising from the thoracic ganglion, after technical grade of diazinon had been used.

Experiments were also done in order to see whether doses having similar lethal effects gave comparable amounts of inhibition in the brain and thoracic ganglion of both strains. When a dose of diazinon (0.4%) causing over 99 per cent. kill was applied to flies of the susceptible strain, considerable inhibition of cholinesterase occurred in both the brain and the thoracic ganglion in less than three hours, when the flies were already dead, and inhibition was complete throughout both organs after 24 hours.

When a dose of 1.0 per cent. diazinon, causing approximately 99 per cent. kill, was applied to the resistant flies, lesser amounts of cholinesterase inhibition had occurred in the thoracic ganglia within three hours than in the thoracic ganglia of

the susceptible flies after a comparable lethal dose. The flies of both strains were apparently dead at this time. Cholinesterase inhibition in the thoracic ganglia of the resistant flies was progressive and after 18–24 hours was fairly extensive. The brains of the resistant flies, however, showed only a small degree of peripheral and suboesophageal inhibition up to 24 hours after poisoning.

These experiments show that cholinesterase inhibition can be correlated with the death of the fly. However, if death is due to cholinesterase inhibition, it must be due to local inhibition since, in dead flies, while there were areas where inhibition appeared to be complete, there were also other areas having considerable cholinesterase activity. When the doses applied to the susceptible and resistant strains are approximately equivalent in their toxic action, it appears that a great deal more inhibition occurs in the susceptible strain than in the resistant strain.

It is not yet possible to say whether one particular area is invariably inhibited in both strains at the time of prostration. However, there is nearly always peripheral inhibition in the brain and thoracic ganglion at this time, and also inhibition in the suboesophageal ganglion and lamina ganglionaris. The region involved in the death of the insect, supposing this to be due to cholinesterase inhibition, may be a small part of one of these areas.

Discussion.

Total cholinesterase inhibition, or even a high percentage of cholinesterase inhibition, is not essential for the death of a house-fly poisoned with diazinon. Considerable amounts of active enzyme are usually present at the time of prostration and for some time after apparent death. Total cholinesterase inhibition throughout the central nervous system only occurs in rare instances some hours after the application of a lethal dose of diazinon. The inhibition of cholinesterase which does occur in the central nervous system is confined to certain localised areas. It is first obvious in the peripheral part of the thoracic ganglion, and almost as consistently, but usually slightly later, in the motor centres of the brain, particularly the suboesophageal ganglion. It also occurs fairly rapidly in the lamina ganglionaris, which is a sensory centre.

These results are corroborated by the observations of Stegwee (1959) and van Asperen (1960a) of low total cholinesterase inhibition at the time of prostration, but cannot easily be reconciled with the results of Smallman & Fisher (1958), Mengle & Casida (1959), Mengle & O'Brien (1960) and Colhoun (1959a), which show a very high percentage of total cholinesterase inhibition at prostration. The histochemical results are, however, more informative than the over-all figures of about 20 per cent. cholinesterase inhibition shown by Stegwee (1959) and van Asperen (1959, 1960a) because they show highly localised areas of complete inhibition of cholinesterase, together with other areas in which no inhibition has occurred.

Gross biochemical methods cannot demonstrate high local inhibition as apart from high generalised inhibition. This limitation does not seem to have been sufficiently realised, and has led to some confusion of thought. Van Asperen (1960a) suggested that his low over-all inhibition values might conceal a high percentage inhibition at some essential part of the nervous system, but he was not able to prove this with the normal biochemical techniques at his disposal. The results reported here show that after diazinon poisoning, cholinesterase inhibition does in fact occur in some parts of the nervous system before it occurs in other parts, and therefore the value of estimating total cholinesterase inhibition is reduced.

With more refined histo- and cytochemical techniques it should be possible to localise the site of action of the poison even more precisely and to make quantitative measurements of enzyme activity in any particular part of the nervous system.

Indications that this highly localised inhibition of cholinesterase is directly connected with the death of the insect are as follows:—(a) Inhibition is usually more pronounced in badly affected flies than in unaffected flies which have received the same dose of poison. (b) There is no ganglionic cholinesterase inhibition among the living flies of a resistant strain, following a dose of diazinon identical with that causing considerable ganglionic inhibition in, and the death of, susceptible flies. (c) When the resistant flies were given 25 times this dose, i.e., a dose causing 99 per cent. kill, cholinesterase was inhibited in the ganglia, even though this occurred to a somewhat lesser extent than in the ganglia of susceptible flies treated with a similar lethal dose.

There is therefore some correlation between cholinesterase inhibition in the ganglia and the death of the insect, but this does not necessarily mean that cholinesterase inhibition is a primary cause of death. Van Asperen (1960a) has pointed out that, after poisoning house-flies with various organophosphorus compounds, there is a very high percentage aliesterase inhibition, in comparison with low percentage cholinesterase inhibition at the time of prostration. This could mean that inhibition of aliesterase is more important than cholinesterase inhibition in causing the death of the fly. On the other hand, Stegwee (1960) showed that considerable aliesterase inhibition in house-flies treated with tri-*o*-tolyl phosphate (TOCP, cited as tri-*o*-cresylphosphate), which is a selective inhibitor not affecting cholinesterase, did not cause death. When these flies were treated 24 hours later with TEPP, typical symptoms of organophosphate poisoning developed. This may indicate that death results from the inhibition of more than one esterase, but the very high values for aliesterase inhibition reported by van Asperen seem to be less important in causing the death of the insect than the much smaller and, as has been shown here, very localised, inhibition of cholinesterase in the central nervous system.

It is interesting that the thoracic ganglion, which controls segmental reflex action, and the suboesophageal ganglion, which excites the thoracic centres and maintains locomotor activity (Roeder, 1953) are both areas which are usually rapidly inhibited by diazinon poisoning. It is therefore not surprising that early indications of poison symptoms are peculiarities in the locomotor activity of the insect, including partial paralysis of the limbs. This is later followed by bursts of hyperactivity and eventual prostration with occasional twitching of the limbs.

Other areas of the brain inhibited shortly after poisoning are the motor and association nerve cell regions (outside the neuropile), the lamina ganglionaris beneath the retinal cells of the large compound eyes, and also parts of the deuto- and tritocerebral lobes, which are both sensory and motor centres for the antennal and mouthpart segments. The lamina ganglionaris is the most noticeable sensory area to be affected; the reason for this is not known. It may be simply that the poison can penetrate into the brain more rapidly here *in vivo*, since the sensory retinal cells are so close to the brain, and might provide a relatively easy pathway into the brain, which is otherwise protected by the usual connective tissue sheath or neurilemma.

The rest of the optic lobes, in particular the medulla interna and the medulla externa, concerned with the co-ordination of optic stimuli may, together with parts of the protocerebrum, still contain large amounts of enzyme even after death.

It seems probable that, if ganglionic cholinesterase inhibition is the cause of death in organophosphorus-poisoned flies, the region involved is in the peripheral part of the ganglia or in the suboesophageal ganglion, as these areas are nearly always noticeably inhibited after death. It is difficult to see how inhibition of an optic centre, such as the lamina ganglionaris, could be a direct cause of death in the insect.

There were indications that reactivation of cholinesterase may occur at some stage after poisoning, possibly after death, since samples of flies examined a long

time after poisoning (24 hours) sometimes showed more enzyme present than samples taken a shorter time after poisoning (two to three hours).

Summary.

The distribution of cholinesterases hydrolysing acetyl- and butyrylthiocholine in the brain and thoracic ganglion of *Musca domestica* L. was examined histochemically in untreated flies, and in flies poisoned with diazinon (O,O-diethyl O-2-isopropyl-4-methyl-6-pyrimidinyl phosphorothioate) of both susceptible and resistant strains.

The inhibition of cholinesterase after poisoning was, to a greater or lesser extent, confined to the peripheral region of the ganglia, and to other specific areas such as the suboesophageal ganglion and lamina ganglionaris. The extent of inhibition increased with increasing doses of poison, and increasing time after treatment. The degree of inhibition could be broadly correlated with the condition of the fly; badly affected or moribund flies having less active cholinesterase than living and unaffected flies. Together with these areas of more or less complete inhibition were other areas, especially in the neuropile or synaptic regions, in which there remained large amounts of active enzyme. Active enzyme was still present in these areas 24 hours after apparent death of the fly. Total inhibition of cholinesterase throughout the central nervous system was rarely seen, even after very high doses of diazinon causing over 99 per cent. kill.

Inhibition did not occur in the ganglia of living flies of a diazinon-resistant strain after a dose which caused inhibition and death in the normal susceptible strain. With 25 times this dose, causing approximately 99 per cent. kill in the resistant flies, cholinesterase was inhibited in them also, although to a somewhat lesser extent than in the susceptible flies given a comparable lethal dose.

The data provided strong evidence that if death is caused by inhibition of cholinesterase of the nervous system it is due to local inhibition and not to generalised inhibition.

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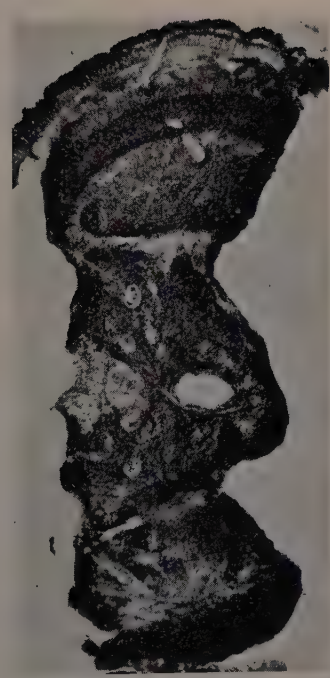


FIG. 1. (Susceptible strain.) Control fly, treated acetone only. Alive. Normal distribution of cholinesterase.

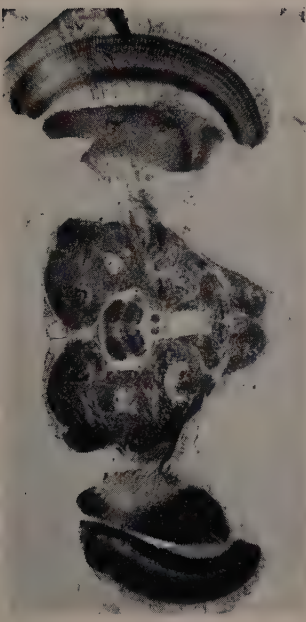


FIG. 2. (Susceptible strain.) Treated 0.04 % diazinon. Prostrate 30 min. after application of poison. Inhibition of cholinesterase in peripheral and neuronal regions. Active enzyme in synaptic regions.

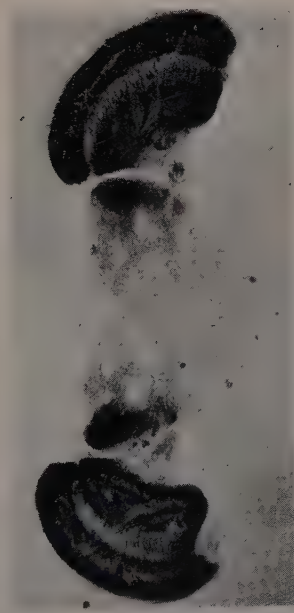


FIG. 3. (Susceptible strain.) Treated 0.04 % diazinon. Badly affected 40 min. after application of poison. Inhibition of cholinesterase in suboesophageal ganglion, deuto- and trito-cerebral lobes, part of the protocerebral lobes and lamina ganglionaris. Active enzyme in dorsal part of protocerebral lobes and in medulla interna and externa of optic lobes.



FIG. 4. (Diazinon-resistant strain.) Treated 0.04 % diazinon. Alive 40 min. after application of poison. No inhibition of cholinesterase.

Frozen sections (25μ) of brain of *Musca domestica*, of strains susceptible and resistant to diazinon, incubated in acetylthiocholine iodide for 20 minutes at 25°C .



FIG. 1. (Susceptible strain.) Control fly, treated acetone only. Alive. Normal distribution of cholinesterase.

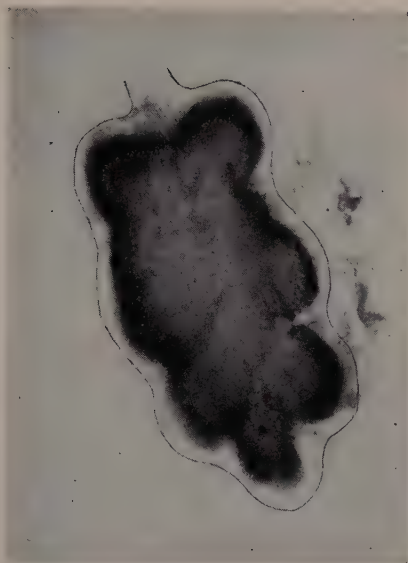


FIG. 2. (Susceptible strain.) Treated 0.015% diazinon. Badly affected 2½ hr. after application of poison. Peripheral inhibition. (N.B. the outline of the ganglion, which did not show well in the photograph, has been drawn in black ink.)



FIG. 3. (Susceptible strain.) Treated 0.015% diazinon. Dead 2½ hr. after application of poison. Further peripheral inhibition of cholinesterase. Neuropile still contains active enzyme.



FIG. 4. (Resistant strain.) Treated 0.04% diazinon. Alive 18 hr. after application of poison. No inhibition of cholinesterase.

Whole thoracic ganglia (fused pro-, meso- and metathoracic ganglia and abdominal ganglia) of *Musca domestica*, of strains susceptible and resistant to diazinon, incubated in acetylthiocholine iodide for 20 minutes (fig. 4 for 10 minutes only) at 25°C.

FURTHER FIELD EXPERIMENTS ON THE CONTROL OF WHEAT BULB FLY, *LEPTOHYLEMYIA COARCTATA* (FALL.).

By F. E. MASKELL

National Agricultural Advisory Service, Cambridge

and

R. GAIR

National Agricultural Advisory Service, Shardlow, Derby.

Field studies on the chemical control of wheat bulb fly, *Leptohylemyia coarctata* (Fall.), have been conducted for a number of years in the eastern half of England. Gough & others (1961) reported the results of experiments up to 1957 and dealt mainly with the effects of insecticides formulated as seed dressings. The present paper covers the period 1957-1959 and is concerned with the combine-drilling of insecticides and their comparison with insecticidal seed dressings.

Gough & others (1961) showed that aldrin and dieldrin dusts combine-drilled at 4 lb. active ingredient per acre gave an effective control of wheat-bulb-fly damage to winter wheat. These rates were quite uneconomic apart from possible deleterious effects on beneficial soil fauna. Moreover the dusts used did not flow easily through the combine-drill, and granular formulations were desirable. A logical development was to use a granular cereal fertiliser as the filler (Kulash, 1955) and study the effect of the fertiliser alone and with insecticide incorporated. The experiments described here were designed to obtain information on the optimum rate of aldrin and dieldrin per acre when combine-drilled.

In most of the trials reported below, combine-drilled treatments were compared with high-level seed dressings already shown to be highly effective against the pest (Gough & Woods, 1954; Bardner, 1958, 1959; Way, 1959). As new materials became available they were tested as seed dressings, combine-drilled granules or as both. The effects of seed dressings formulated from mixtures of insecticides were also studied.

Site details for experiments in both Eastern and East Midland Regions of the National Agricultural Advisory Service are given in Table I. Details of the layout and methods of assessment of treatment effects were identical with those used in the earlier series (Gough & others, 1961). Other details of individual experiments are given for each year, together with the reasons for the choice of treatments. All the results are discussed together later.

The 1957 trials.

At the suggestion of Mr. J. A. Birch, crushed brick was used as an inert base; this was screened to a suitable size and impregnated with aldrin or dieldrin at two rates such that 2 or 4 lb. active ingredient was applied in $1\frac{1}{2}$ cwt. material per acre. These were compared with superphosphate granules impregnated with aldrin and a compound potato fertiliser containing aldrin, both of which were tested in Expt. 3.

Treatment details for Expts. 1-5, together with assessment data, are given in Table II.

It was not possible to combine-drill the exact amount of brick granules, the high

TABLE I.
Details of trial sites, 1957-59.

Expt. No.	Site/County	Soil type	Date of drilling	Wheat variety	No. of larvae 1000s/acre in control plots	Treatments \times replications in randomised blocks
1	Cavendish, Suffolk	Clay loam	22.x.56	Cappelle	1,120	8 \times 4
2	Newborough, Soke of Peterborough	Peat	31.x.56	"	434	8 \times 4
3	Ashdon, Essex	Clay loam	27.xi.56	Heme VII	342	5 \times 6
4	Misson, Notts.	Sand	8.xi.56	Cappelle	1,139	10 \times 4
5	Thistleton, Rutland	Clay loam	16.xi.56	"	299	12 \times 5
6	Radwinter, Essex	"	16/21.x.57	"	1,341	12 \times 4
7	Birdbrook, Essex	"	7.xi.57	"	866	12 \times 4
8	King's Cliffe, Northants.	"	15.xi.57	"	234	10 \times 4
9	Belchamp Walter, Essex	"	17.xi.58	"	1,057	9 \times 4
10	Abbotsley, Hunts.	Peat	18.xi.58	"	1,200	10 \times 4
11	Peterborough, Soke of Peterborough	Clay loam	19.xi.58	"	1,234	9 \times 4
12	Warrington, Northants.	"	24.xi.58	"	501	10 \times 4

TABLE II.
Summary of results of seed-dressing and combine-drilled trials, 1957.

Treatment	Expt. 1		Expt. 2		Expt. 3		Expt. 4		Expt. 5		
	live larvae Mar. % Ang.	shoot attack Apr. % Ang.	Yield (cwt./ acre) (15% M)	live larvae Mar. % Ang.	shoot attack Apr. % Ang.	Yield (cwt./ acre) (15% M)	live larvae Mar. % Ang.	shoot attack Apr. % Ang.	Yield (cwt./ acre) (15% M)	shoot attack Apr. % Ang.	Yield (cwt./ acre) (15% M)
Seed dressings (2 oz./ bushel)											
40% γ BHC + o.m.	83 67	23 32	28.1	89 74	32 34	41.1		58 50	15.8	35 37	24.7
60% dieldrin + o.m.	64 55	17 25	32.1	78 68	26 30	39.9				39 40	24.4
40% dieldrin + o.m.										40 39	24.6
Organo-mercury control	92 74	40 37	15.4	92 74	42 41	42.6	91 78	80/81 63/64	9.1	31 34	24.5
Combine-drilled at 1½ cwt./acre											
Brick + 2 lb. aldrin/acre	68 55	20 27	24.7	89 71	32 35	39.7	44 41	47 44	18.8	44 41	24.1
Brick + 4 lb. aldrin/acre	75 62	19 26	27.3	90 76	31 33	41.3		60 51	16.7	38 40	26.8
4% aldrin dust at 2 lb.											
aldrin/acre								40 39	24.4	44 41	23.9
Brick + 2 lb. dieldrin/acre	76 62	22 28	24.0	92 75	33 35	40.2		74 60	10.7	36 37	24.6
Brick + 4 lb. dieldrin/acre	69 56	19 26	26.7	91 73	37 37	41.3		75 62	14.6		
4% dieldrin dust at 2 lb.								41 40	23.1	40 39	23.3
dieldrin/acre								44 41	20.3	34 35	24.5
Brick control	90 72	49 45	18.3	92 79	33 35	42.1				37 37	27.6
1½% dieldrin dust at											
14% lb. dieldrin/acre										39 39	23.8
14% aldrin dust at 2½ lb.											
aldrin/acre											
Superphosphate + 2 lb.							51 45	13 21	15.5		
aldrin/acre							99 87	19 25	10.9		
Superphosphate control											
Potato fertiliser + 0.3lb.							53 47	12 20	14.9		
aldrin/acre											
S.E. of treatment mean	±4.5	±2.3	(a) ±1.37 (b) ±1.69	±5.0	±1.2	±2.35	±3.4	±0.8	±0.63	±2.7	±1.70
										±2.1	±1.31

S.E. (a) for combine-drilled treatments and control.

S.E. (b) for seed-dressing treatments.

M = Moisture.

o.m. = organo-mercury.

Ang. = Angular transformation.

TABLE III.
Summary of results of seed-dressing and combine-drilled treatments, 1958.

Treatment	Expt. 6				Expt. 7				Expt. 8			
	% live larvae March	Ang. transf. March	% shoot attack March	Ang. transf. March	Yield (cwt./acre) (15% M)	% live larvae March	Ang. transf. March	% shoot attack April	Ang. transf. April	% live larvae March	Ang. transf. March	% shoot attack April
Seed dressings (2 oz./bushel)												
40% heptachlor + o.m.	81	64	37	37	30.4	62	47	27	32	71	57	15
40% γ-BHC + o.m.	58	50	21	28	23
40% γ-BHC only	23	29	23	29
60% dieldrin + o.m.	83	65	81	65
Organo-mercury control
Combine-drilled												
Granular clay + 0.5 lb. aldrin/acre	87	70	33	36	32.3	48	42	48	44	82	65	19
Fertiliser + 0.5 lb. aldrin/acre	88	70	32	34	33.7	56	51	40	39	59	50	24
Fertiliser + 1.0 lb. aldrin/acre	74	59	31	34	34.2	29
*Granular clay + 1.8, 2.4 lb. aldrin/acre	82	67	32	34	33.3	42	44	26	33	60	51	16
*Fertiliser + 1.8, 2.4 lb. aldrin/acre	71	57	31	34	33.8	33	33	31	35	60	51	11
*Granular clay + 2.6, 3.3, 4 lb. aldrin/acre	61	52	39	38	33.6	44	42	27	32	80	44	11
*Fertiliser + 3.1, 2.9, 4.4 lb. aldrin/acre	61	52	29	33	34.1	36	37	25	31	84	53	11
Granular clay only	92	75	33	35	32.4	79	62	78	62	73	60	32
Fertiliser only	93	75	31	34	32.6	82	65	71	58	82	63	36
2% aldrin dust at 2.4 lb. aldrin/acre	78	62	18
S.E. of treatment mean	± 3.8	± 1.0	± 0.33	± 6.0	± 2.1	± 1.08	± 4.8	± 0.9	± 0.52

*The actual amounts of aldrin applied at the medium (nominal 2 lb.) and high (nominal 4 lb.) levels on each site are given in the following order : Expt. 6, 7, 8.
All seed for combine-drilled treatments dressed with organo-mercury at 2 oz./bushel.
M = Moisture.

rate of aldrin varying from 4–5½ lb. and the low rate from 2–3 lb. active ingredient per acre in the different trials.

In Expt. 1, a drilling error resulted in the combine-drilled treatments and organo-mercury controls being drilled at a seed-rate of 1½ bushels per acre and the remaining plots at 2½ bushels per acre. At site 3, the phosphate status of the soil was very low; in January 1957, severe rabbit grazing was noted on the plots, and although the whole site was wired on 1st February the plants received a severe setback which partly accounted for the low yields. At Thistleton (Expt. 5) the expected heavy tiller attack by wheat-bulb-fly larvae developed on only one of the treatment blocks, plants on the remainder of the trial being virtually unaffected. Plant establishment counts revealed no adverse effects on germination by any of the treatments employed.

It should be noted that because aldrin and dieldrin gave closely comparable results when combine-drilled, and because aldrin was much cheaper, further work was confined to this insecticide.

The 1958 trials.

The crushed brick granules used in the previous year gave an anomalous result in Expt. 4 and were found generally unsatisfactory for combine-drilling because of their hardness. Messrs. Fisons Ltd. kindly provided an alternative material in the form of granular clay with varying amounts of impregnated aldrin, together with the same range of aldrin added to a compound fertiliser (5:12½:12½). The intention was to apply ½, 2 or 4 lb. actual aldrin per acre when combine-drilling clay base or fertiliser at 3 cwt. per acre; as in 1957, however, this was found difficult to achieve and the actual rates of aldrin applied are shown in Table III, which gives full treatment details and assessments of their effects.

Mr. H. M. Fox (*in litt.*) had suggested that the organo-mercury component of dual-purpose seed dressings containing γ BHC might be enhancing the effect of the insecticide. A seed dressing of 40 per cent. γ BHC only was therefore included in Expt. 7 for comparison with one containing organo-mercury as well.

TABLE IV.

Summary of results of Expt. 9—commercial products trial, Belchamp Walter, 1959.

Treatment	% live larvae April	Ang. transf.	% shoot attack April	Ang. transf.	Yield (cwt./acre) (15% M)
Seed dressings (2 oz./bushel)					
40% γ BHC + o.m.	74	61	23	29	36.2
60% dieldrin + o.m.	11	20	21	28	37.8
40% aldrin + o.m.	5	11	22	28	37.3
40% heptachlor + o.m.	1	3	18	25	38.9
Organo-mercury control	88	70	70	57	15.9
Combine-drilled at 3 cwt./acre					
Superphosphate + 1 lb. aldrin/acre ..	N/A		N/A		26.7
Fertiliser (6 : 15 : 15) + 1 lb. aldrin/acre	21	30	19	27	37.4
Superphosphate only	N/A		N/A		13.8
Fertiliser (6 : 15 : 15) only	N/A		N/A		16.9
S.E. of treatment mean	± 4.3		± 1.8		± 1.26

All seed-dressing treatments except control combine-drilled with 3 cwt./acre fertiliser (6:15:15). Seed for combine-drilled treatments dressed with organo-mercury at 2 oz./bushel.

N/A=no assessment made.

M=Moisture.

TABLE V.
Summary of results of Expt. 10—combine-drilling trial, Abbotsley, 1959.

Treatment	% live larvae March	Ang. transf.	% shoot attack April	Ang. transf.	Yield (cwt./acre) (15% M)
1 cwt./acre superphosphate + 1 lb. aldrin/acre	27	31	4	12	31.5
2 cwt./acre superphosphate + 2 lb. aldrin/acre	17	25	3	10	34.2
1 cwt./acre superphosphate + 1 lb. aldrin/acre + 40% γ BHC S.D.	32	34	4	12	34.1
2 cwt./acre superphosphate + 2 lb. aldrin/acre + 40% γ BHC S.D.	8	18	2	7	36.2
3 cwt./acre fertiliser (6 : 15 : 15) + 1 lb. aldrin/acre	35	36	9	17	31.8
3 cwt./acre fertiliser (6 : 15 : 15) + 1 lb. aldrin/acre + 60% dieldrin S.D.	24	30	4	11	35.3
2 cwt./acre superphosphate + 2 lb. heptachlor/acre	10	17	2	8	37.4
2 cwt./acre superphosphate only	88	70	41	43	16.4
3 cwt./acre fertiliser (6 : 15 : 15) only	86	68	33	39	12.4
Organo-mercury control	91	74	57	52	13.4
S.E. of treatment mean		\pm 4.0		\pm 4.4	\pm 0.99

Seed for all treatments dressed with organo-mercury.

S.D. = seed dressing.

M = Moisture.

TABLE VI.
Summary of results of Expts. 11 and 12, Peterborough and Warrington, 1959.

Treatment	Expt. 11			Expt. 12		
	% live larvae March	Ang. transf.	% shoot attack March	Ang. transf.	No. live larvae per 2ft. sample March	% shoot attack March
Seed dressings (applied with sticker)
60% Thiodan at 2 oz./bushel	55	49	59	51	3.3	37
60% Thiodan at 10 oz./bushel	28	32	44	42	0.9	42
60% dieldrin at 2 oz./bushel	64	54	66	54	1.4	35
60% dieldrin at 10 oz./bushel	22	26	47	43	0.1	39
60% heptachlor at 2 oz./bushel	64	55	74	60	5.4	33
60% heptachlor at 10 oz./bushel	18	25	38	38	0.5	38
40% γ BHC at 2 oz./bushel	49	46	73	59	11.5	37
Mixture 40% γ BHC/40% dieldrin at 2 oz./bushel	38	38	51	45	12.2	40
Organo-mercury control	89	71	85	67
Combine-drilled
3 cwt./acre superphosphate + 1.2 lb. aldrin/acre
3 cwt./acre superphosphate only
S.E. of treatment mean	± 5.1	± 1.5	± 0.93	± 2.4	± 2.09	± 2.09

Seed for all treatments dressed with organo-mercury.
M = Moisture.

The soil phosphate status was low at each site. Plant establishment counts on Expt. 7 showed that dual-purpose seed dressings of γ BHC and dieldrin had adversely affected germination. At the earlier sown trial at Radwinter (Expt. 6), no such differences were at first recorded, but subsequently all seed-dressing plots were visibly poorer than combine-drilled ones. In Expt. 8, the combine-drilled treatment of compound fertiliser containing 4.4 lb. actual aldrin per acre visibly delayed brairding, but this effect disappeared after some weeks.

The 1959 trials.

In Experiment 10, aldrin at 1 or 2 lb. per acre and heptachlor at 2 lb. per acre in a superphosphate base were combine-drilled with seed treated with organo-mercury alone or with a dual-purpose seed dressing containing dieldrin or γ BHC. In Expts. 11 and 12, the effect of aldrin combine-drilled at 1 lb. per acre on a superphosphate base was compared with seed dressings containing insecticide at 2 or 10 oz. per bushel and which were applied to the seed by means of an aqueous solution of methyl cellulose (Bardner, 1958). A number of materials then commercially available for control of wheat bulb fly were tested at Belchamp Walter (Expt. 9).

Full details of treatments in Expts. 9-12 and the assessments of their effects are shown in Table IV (Expt. 9), Table V (Expt. 10) and Table VI (Expts. 11 and 12).

The phosphate status on all clay loam soils was low. At Peterborough (Expt. 11), the potash status was also low and the pH was 6.2.

No phytotoxic effects were observed on Expt. 9 or 10. At both Peterborough and Warmington (Expts. 11 and 12) severe phytotoxic effects were soon apparent on those plots with the high rate of insecticidal seed dressing; this was confirmed in subsequent germination tests on surplus seed. Because of an error in formulation, the actual quantities of organo-mercury and insecticide applied to the seed were double those of the intended 5 oz. rate. The wheat at Warmington recovered sufficiently for harvest yields to be taken but at Peterborough the high-rate plots failed, and the low rates of seed dressing did not protect the plants from wheat-bulb-fly attack. The whole trial was therefore abandoned in April.

Discussion.

The value of early drilling of winter wheat to withstand wheat-bulb-fly damage has been repeatedly stressed (*e.g.*, Petherbridge, Stapley & Wood, 1945). Further evidence of its importance is obtained from a comparison of Expts. 6 and 7, which were drilled on similar soil types and with comparable autumnal egg populations. At Radwinter (Expt. 6), drilled three weeks before Expt. 7 at Birdbrook, there was a higher number of shoots per plant in early spring and hence a lower percentage attack with little effect on yield. This difference in shoot numbers could easily account for the higher number of larvae per acre in Expt. 6, since only one live larva is usually found in each attacked shoot and the number of available shoots limits the number of larvae surviving to maturity (Raw, 1960).

None of the chemical treatments used in the trials reported above afforded complete protection from attack by wheat-bulb-fly larvae. However, the trials were deliberately sited on fields having high autumnal egg populations and the plots were often drilled late to encourage heavy damage. In these adverse circumstances, chemical control methods gave some, and usually a great, improvement upon untreated controls. In trials where the number of larvae per acre in the control plots was well over 500,000, the differences in yield between treatment and no treatment was usually greater the later the field was sown (Expts. 4, 7, 9 and 10). This agrees with the earlier work of Gough and others (1961).

The similarity between aldrin and dieldrin when combine-drilled (Table II) has already been mentioned. The optimum rate of aldrin for wheat-bulb-fly control is

rather difficult to conclude from the experiments conducted. In Expt. 3, with a low larval count on the controls, aldrin at 0.3 lb. per acre gave a surprisingly good result. In Expt. 6, the treatment effects were masked by early drilling, and in Expt. 8 there was a low attack. In Expt. 7, with a heavy attack and fairly late drilling for a clay soil, the protection from attack at rates much below 1 lb. per acre was significantly poorer than that provided by 1.8 lb. per acre and above. Bearing all these points in mind it would seem that the optimum rate is probably around 1 lb. of aldrin per acre. The slight advantages gained from applying aldrin at rates much higher than 2 lb. per acre (Tables II, III) would be more than offset by increased costs, apart from the difficulties in formulating such materials. Such differences as occurred between 1 and 2 lb. actual aldrin per acre (Table V) would probably be much less on fields with low to moderate egg populations. It was interesting to note that heptachlor when combine-drilled at 2 lb. active ingredient per acre (Table V) was slightly superior to aldrin at the same rate.

The choice of inert base materials impregnated with insecticide in a condition suitable for combine-drilling presented some difficulties. The fine dusts of aldrin and dieldrin normally used for broadcast treatments against soil pests, and which had been used in the earlier combine-drilling experiments (Gough & others, 1961), were again highly effective against the pest (Table II) but did not flow easily through the drill. Crushed brick was not a satisfactory alternative.

Addition of a fertiliser at various levels of incorporated aldrin (Tables II, III) had virtually no effect upon wheat-bulb-fly attack but generally resulted in higher yields. The total shoot count on the trials in Table III showed a 5 per cent. increase in the treatments receiving fertiliser compared with those with no fertiliser.

Turning to seed-dressing treatments, which were generally slightly more effective than combine-drilling, dieldrin and heptachlor gave consistently satisfactory results (Tables II, III, IV, VI) under a wide range of conditions, with aldrin and Thiodan seed dressings (Tables IV, VI) almost as effective. In most experiments, a commercial preparation of γ BHC was almost equal in effectiveness to the best alternative seed dressings, whereas in Expt. 12 (Table VI), using an experimental formulation, it was markedly inferior. The mixture seed dressing of γ BHC and dieldrin gave promising results in Expt. 11 (Table VI) and indicates a possible future development in seed-dressing formulation. The results of Expt. 7 (Table III) do not support the contention that organo-mercury enhances the effectiveness of γ BHC.

Counts of the numbers of live larvae within attacked shoots throw light on the mode of action of these insecticidal seed dressings and confirm the opinion of previous workers (Gough & Woods, 1954; Bardner, 1958; Way, 1959). Dieldrin, aldrin, heptachlor and Thiodan allow larvae to enter the wheat plant but kill a high proportion of them effectively afterwards; γ BHC reduces the number of larvae entering the plant, but those which succeed in penetrating feed in an apparently normal manner. The foregoing remarks concern insecticidal seed dressings applied at the normal rate of 2 oz. per bushel of 40 or 60 per cent. material. In Expts. 11 and 12 (Table VI) much higher rates of 60 per cent. insecticide were accidentally applied in the high-level seed-dressing treatments, and the reduction of numbers of live larvae at both sites was even more striking. Symptoms of phytotoxicity resulting from seed-dressing treatments were observed following these overdoses of insecticide and organo-mercury and, except for heptachlor, these high rates decreased yields in Expt. 12, a reflection of the early setback to the crop. In Expt. 6, all seed-dressing treatments were visibly poorer throughout the season, while in Expt. 7 both γ BHC and dieldrin seed dressings adversely affected seed germination. In all other cases, seed treated with dual-purpose dressings germinated in an apparently normal manner.

Slight additional improvements were obtained when wheat dressed with either γ BHC or dieldrin was combine-drilled with aldrin (Table V), and no symptoms of phytotoxicity were recorded. Such double treatment is justifiable only where a very heavy infestation is known or expected. Under normal conditions, seed dressings alone form an effective control of wheat-bulb-fly damage, the choice of insecticide depending on relative costs and other factors. The use of seed dressings has the advantage of applying only small amounts of insecticide to the soil. Combine-drilling of approximately 1 lb. actual aldrin per acre, preferably on a fertiliser base, provides an effective alternative when the seed has been treated with an organo-mercury fungicide only.

Summary.

Field trials were conducted in certain eastern counties of England in the years 1957-1959 to assess chemical control methods applied at sowing time against wheat bulb fly, *Leptohylemyia coarctata* (Fall.). Combine-drilled treatments, using fillers of aluminium silicate, brick dust, granular clay, superphosphate or compound fertiliser, included aldrin at rates from 0.3 to 5.5 lb., dieldrin at 2 and 4 lb., and heptachlor at 2 lb. active ingredient per acre, respectively. Seed-dressing treatments applied with organo-mercury fungicide included 40 and 60 per cent. dieldrin and heptachlor, 40 per cent. aldrin, 60 per cent. Thiodan, 40 per cent. γ BHC with and without organo-mercury, all applied at 2 oz. per bushel of seed; 60 per cent. dieldrin, heptachlor and Thiodan were also tested at double the intended rate of 5 oz. per bushel.

No form of chemical control was completely effective in suppressing damage but all gave some, and usually a great, improvement, particularly on late-sown or backward crops. Most of the insecticides tested gave closely comparable results. Seed dressings containing at least 40 per cent. heptachlor, dieldrin, aldrin or γ BHC were slightly more effective than combine-drilled insecticidal treatments, with the added advantage of applying only relatively small amounts of persistent insecticides to the soil.

Slight symptoms of phytotoxicity were observed on two sites in 1958 following the use of γ BHC and dieldrin seed dressings applied at the normal rate of 2 oz. per bushel and severe symptoms on two trials in 1959 to seed over-dressed with insecticide and organo-mercury; elsewhere the seed dressings employed appeared to have no adverse effect upon plant establishment.

Dieldrin, aldrin, heptachlor and Thiodan seed dressings behaved similarly in killing a high proportion of larvae within attacked shoots; γ BHC reduced the number of larvae entering the plant, but those which did succeed in entering developed in an apparently normal manner.

On fields where drilling was delayed and the attack severe, the optimum rate of combine-drilled aldrin was probably between 1 and 2 lb. active ingredient per acre. No significant increase in yield was obtained at rates much higher than 2 lb. per acre. While fine-dust formulations gave effective results, the use of a granular fertiliser base improved the flow through the combine-drill and gave increased tillering with slightly higher yields. Heptachlor combine-drilled at 2 lb. active ingredient per acre was slightly superior to aldrin at the same rate.

No adverse effects were recorded when insecticidal seed dressings and combine-drilled aldrin were used together, and at high levels of infestation the double treatment gave increased yields, though insufficient to justify its use on fields having only moderate egg populations.

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THE BIOLOGICAL SIGNIFICANCE OF THE ATTACHMENT OF IMMATURE STAGES OF *SIMULIUM* TO MAYFLIES AND CRABS.

By PHILIP S. CORBET

East African Virus Research Institute, Entebbe, Uganda.

The attachment of the immature stages of SIMULIIDAE to other aquatic arthropods was evidently first noticed about 20 years before the association was regarded as other than fortuitous. In 1926, Ribeiro reported the finding of a Simuliid larva attached to a mayfly larva which had been collected in the eastern Himalayas, and remarked that the association was apparently accidental. Soon afterwards, F. W. Edwards (1928) recorded the occurrence of larvae and pupae of *Simulium nyasalandicum* De Meillon (referred to by him as *S. hirsutum* Pomeroy) on a specimen of the crab, *Potamon niloticum* (H. M.-Edw.), collected in Uganda, and likewise concluded that the attachment must have been fortuitous. And in 1929, W. N. Edwards collected from the Victoria Falls a mayfly larva of the genus *Afronurus* to which were attached the pupa and cocoon of a *Simulium*, possibly *S. lumbwanus* De Meillon (see Freeman & De Meillon, 1953).

The specificity of the relationship was apparently recorded first by Rubtsov (1948, quoted by Grenier & Mouchet, 1958), who described the attachment of *S. ephemerophilum* Rubtsov to larvae of *Ecdyonurus* in Turkestan. Subsequently, and independently, Marlier (1950) and van Someren & McMahon (1950) reported similar associations with mayflies in West and East Africa, where several other examples have since been recorded (Freeman & De Meillon, 1953; Berner, 1954; M. T. Gillies, personal communication, 1958; Corbet, 1960). The specific attachment of members of the complex of *S. neavei* Roub. to freshwater crabs in Africa, noted by van Someren & McMahon (1950) and later by others seems to represent a relationship which is ecologically similar.

Several authors, particularly Rubtsov, have discussed the possible biological significance of these associations, and have suggested various respects in which the *Simulium* might be expected to benefit from them. This subject has recently been reviewed by Grenier & Mouchet (1958), who have provided a useful summary of the available information. It is my view, however, that, although sufficient evidence necessary for a tentative solution of the problem has now been published, the most plausible of the permissible inferences has not yet been drawn from it, and the purpose of the present communication is to call attention to the one which I consider the evidence unequivocally supports, and then to discuss some of its implications. For conciseness, I shall confine the detailed arguments to the association involving *Simulium* and mayflies, and then briefly explain why essentially the same considerations apply when crabs are involved.

Characteristic features of the association are that larvae or pupae of *Simulium* are attached, in a more or less constant position, to the larva of a mayfly which lives amongst stones on the bed of a fast-flowing stream or river. Species of *Simulium* exhibiting this habit usually attach themselves only to those mayfly larvae which are dorso-ventrally flattened, and which cling tightly to the stones amongst which they live. Examples of such mayflies are members of the HEPTAGENIIDAE (*Afronurus*, *Ecdyonurus*) and OLIGONEURIDAE (*Elassoneuria*).

It was Rubtsov who pointed out that in Turkestan the majority of attached examples of *Simulium* are either pupae or large larvae; and subsequent enquiry and examination have shown that this is true elsewhere. To my mind, this

indicates that the biological significance of the association is to be found in some adaptive requirement peculiar to *older larvae* or to *pupae*, since otherwise it is presumably the *smallest* larvae that would be the most numerous. One thing with which, in their different ways, both large larvae and pupae are concerned, is the location of a suitable site for pupation. Thus the most likely explanation seems to be that the pupa is the stage towards which the adaptation is primarily directed. *A priori* this is equally probable. Being immobile in an environment where the stones of the river bed may frequently be changing position owing to violent water movement, the *Simulium* pupa has need of a support which will (a) adopt a constant orientation with respect to the current, and (b) reduce the chances of its being crushed or buried when stones are displaced. As Rubtsov remarked, certain lithophilic mayfly larvae, which tend both to face the current and to seek sheltered crevices, answer these requirements well. Furthermore, it is evident that the ones most suitable will be those which have a dorsal surface sufficiently large and even to accommodate the pupal cocoon.

Hitherto, this interpretation has been mentioned as merely one of several advantages that the *Simulium* might derive from the relationship. In the light of the evidence, however, I consider that it is more appropriately to be regarded as the principal selective factor which has determined the association. Certainly, such a hypothesis simplifies a problem which writers hitherto have been inclined to regard as complex. It resolves, in particular, the confusing question of whether or not the *Simulium* larva *per se* benefits from the association. Previously it had been suggested that, in an environment where organic matter in suspension was scarce, the larvae might obtain significantly more food by exploiting the detritus dislodged by the mayfly or crab, and also that, as a result of the attachment, they might obtain shelter from the current and enjoy enhanced opportunities for respiration. If, however, the pupa provides the *raison d'être* of the association, then, as explained above, the presence of attached larvae is to be expected simply on the grounds that it is they who must select the pupation site. Were attachment to have any selective value for the early stages, one would expect *Simulium* larvae to be represented in proportion to their abundance—the smallest being the most numerous—whereas in fact the reverse is the case.

To sum up, I consider the evidence supports the hypothesis that the determining factor in the evolution of this association has been the need for *Simulium* in fast-flowing, unstable watercourses to secure a pupation site which gives protection against disorientation and damage.

In the case of associations involving *Simulium* and crabs, the evidence is similar and leads to the same conclusion. The associations usually occur in swiftly flowing streams or rivers, and the attached examples of *Simulium* comprise mainly pupae and large larvae. If, as sometimes happens, small larvae are represented, then they are disproportionately rare (see Browne, 1960). Like certain mayflies, crabs present a smooth, even surface for pupal attachment, and also presumably give adequate protection against disorientation and damage. It should perhaps be stressed here that physical damage due to displacement of stones is more than a theoretical possibility in some habitats. Barnley (1960) noticed that, after heavy rainfall had caused abrupt flooding in highland streams in Uganda, many of the *Simulium* pupae found on the stones were crushed or dead.

It has been suggested that, where streams are liable to dry up or become otherwise unsuitable, crabs may assist the *Simulium* larvae by transporting them across land to a more favourable habitat (see Lewis, 1960). Once again, however, the size-distribution of the attached examples of *Simulium* militates against this being more than a secondary advantage of the relationship, although it is possible that in some habitats selection pressure may now be acting upon it in this way. When this happens to a significant extent, however, we may expect to find small larvae attached in greater numbers.

A second, and separate question may now be considered, namely the means by which *Simulium* larvae locate and select their carriers, and then become attached to them.

The location of a mayfly larva, as such, may perhaps depend upon the slight gradient in water velocity produced by the activity of its abdominal gills. Heptageniid mayfly larvae commonly live beneath stones, in relatively sheltered sites, the dorso-ventral flattening of their bodies being regarded as a crevice-seeking, and not a current-resisting, adaptation (Nielsen, 1951; Stuart, 1958). In such places the contrast between their respiratory current and the water movement of their immediate surroundings might be enhanced, and thus become discernible to a searching *Simulium* larva. Water velocity is known to be one of the most important physical factors determining the micro-distribution of *Simulium* larvae in watercourses (Phillipson, 1956; 1957), and it would therefore seem possible that *Simulium* larvae seeking an attachment site might be able to locate a mayfly larva by responding to short-range current gradients set up by its gill movements.

The point has already been made that most of the attached *Simulium* larvae are large ones. But it can be seen that there is no obvious reason why larvae should not attach themselves in instars other than the final one. Indeed, by exploiting a premature encounter, they would extend their chances of finding a suitable mayfly. To judge from the material of several species I have examined in the British Museum (Natural History) and elsewhere, a few larvae begin to attach when they are about half grown, and others do so in increasing numbers after this. It must be borne in mind, however, that attachment need not be irrevocable, and that, as the time for pupation approaches, attached larvae may perhaps become more critical and may change their supports.

If attachment is to be an insurance against displacement during the pupal stage, then it is clear that the mayfly larva selected as a carrier should neither be about to moult nor about to emerge, since in either event the *Simulium* pupa would then be stranded on the mayfly exuvia and in all probability be swept downstream. Thus the mayfly larvae most suitable for attachment in this respect would be those which had just entered a late instar, *i.e.*, one with a relatively long duration, though not necessarily the last (see Corbet, 1960). There are two physical attributes which are peculiar to such mayfly larvae, and which accordingly might enable *Simulium* larvae to recognise them. These are first, a soft cuticle; and second, a relatively large, even, dorsal surface. It would be interesting to discover whether searching *Simulium* larvae (especially those in the last instar) respond positively to objects possessing these two properties.

It was originally believed that the emergence of the *Simulium* and that of its mayfly carrier were closely synchronised, a conclusion based on the observation that the emergence of captive *Simulium copleyi* Gibbins took place within one or two hours of that of the *Afronurus* carrying them (van Someren & McMahon, 1950). It has recently been shown, however, that so long as the adult of *Simulium* can emerge before the moult of its carrier, no synchronisation of this kind is necessary, a conclusion amply supported by the finding of empty pupae attached to penultimate-instar mayfly larvae (Corbet, 1960). To obtain final confirmation of this, I examined the preserved material of attached *Simulium* in the British Museum (Natural History), and found that the majority of pupae or pupal exuviae of *Simulium* (including a specimen of *S. copleyi*—the species in which van Someren & McMahon reported synchronisation to occur) were attached to mayfly larvae in the penultimate instar. It may be concluded that grounds no longer exist for regarding synchronisation of emergence as a feature of this association.

Most of the remarks above have equal force when applied in principle to the association involving *Simulium* and crabs. *Simulium* larvae could probably locate and recognise crabs in similar ways; and here also it would be advantageous for

a larva seeking a pupation site to select a crab which was at the beginning of an instar (*viz.*, a crab with a soft cuticle), although there would evidently not be the same need for the instar to be a late one. What little information exists indicates that all but the earliest instars of crabs have a duration many times longer than that of the pupal stage of *Simulium*. Hence, whereas it might still be necessary for the *Simulium* larva to select a crab of above a certain size (a phenomenon noted recently by Browne (1960) in *S. neavei*), the range of suitable instars available to it would presumably be much greater than is the case in the mayfly. A recent observation which may be of great significance in relation to this question has been made by Barnley (1960), who remarked that final-instar larvae and pupae of *Simulium* predominated on soft crabs.

If the attachment of *Simulium* to freshwater arthropods has the biological significance which has been attributed to it in this paper, then it is obviously appropriate to describe the association as phoresis.

Summary.

It is suggested that the attachment of the immature stages of certain species of SIMULIIDAE to mayfly larvae and to crabs is primarily an adaptation whereby the immobile *Simulium* pupa can obtain protection against disorientation with respect to the current, and against damage, in an environment where available inorganic substrata are liable to be displaced by violent water movement.

The principal evidence for this is that these associations typically occur in fast-flowing streams or rivers, and that the attached stages of *Simulium* include a disproportionately high number of pupae and large larvae. Any direct benefit the *Simulium* larvae themselves may derive from the association is regarded as a secondary feature of it.

Certain responses to physical factors, which might be expected in *Simulium* larvae achieving successful attachment, are briefly discussed.

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THE EFFECT OF RADIOACTIVE PHOSPHORUS ON THE GROWTH AND
DEVELOPMENT OF *CULEX PIPIENS MOLESTUS* FORSK.
(DIPTERA, CULICIDAE).

By ALBERT A. ABDEL-MALEK *

Dept. of Entomology, Faculty of Science, Cairo University,
Giza, U.A.R.

The use of radioisotopes as tracers for the study of flight ranges of mosquitos has necessitated laboratory studies to ascertain appropriate, safe and efficient methods of utilisation. Preliminary results were published by Hassett & Jenkins (1949) and by Bugher & Taylor (1949) for *Aedes aegypti* (L.) and for arctic species by Jenkins & Hassett (1951). These papers describe early laboratory results and the first field studies for both arctic and tropical species of mosquitos. Abdel-Malek & Abdel-Wahab (1961) studied the uptake of ^{32}P by *Culex pipiens molestus* Forsk. using an autoradiographic technique. In the course of that study, the senior author noticed some effects on the growth and development of the larvae, pupae and adults which were produced by the radioisotope. Laboratory experiments were therefore carried out to ascertain more precisely the effects of radio-phosphorus on *C. p. molestus*. The present paper is a report on the results of these studies.

Materials and methods.

Radioactive larvae, pupae and adults of *C. p. molestus* were produced in the laboratory by putting radioactive phosphorus in the form of $\text{Na}_2\text{H}^{32}\text{PO}_4$ into the larval rearing medium which consisted of tap water and pellets of dog food. The larvae were reared in 100-ml. beakers. Two series of these beakers, labelled I and II, were used, each consisting of seven beakers containing concentrations of ^{32}P as listed in Table I. Fifty ml. of each of these concentrations were made and into each vessel were introduced 100 second-stage larvae of *C. p. molestus*. The laboratory temperature was $17 \pm 2^\circ\text{C}$., and the relative humidity 75 ± 5 per cent.

TABLE I.

Concentrations of ^{32}P used (in microcuries per millilitre per beaker).

Beaker	A	B	C	D	E	F	G
^{32}P concentration in $\mu\text{c./ml.}$	0	0.05	0.5	1.0	3.0	5.0	10.0

From series I, four batches, each of 10 larvae, were removed after 5, 10, 15 and 20-day periods, respectively. The larvae in series II were allowed to reach the adult stage. The larvae removed from series I were killed by being dipped in hot water. They were then washed in three changes of distilled water and then once with 70 per cent. ethyl alcohol before they were stored in 70 per cent. alcohol

* Post-doctorate Fellow, Dept. of Entomology, Macdonald College of McGill University, Ste. Anne de Bellevue, P.Q., Canada.

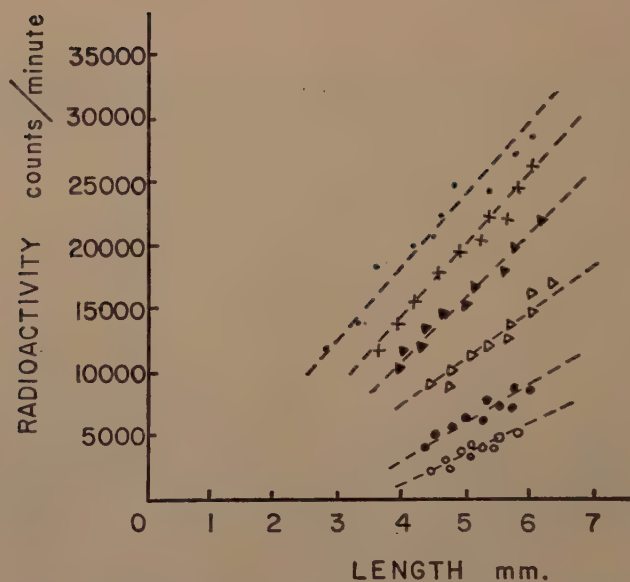


Fig. 1.—Length of larvae of *C. p. molestus* 5 days old, plotted against their radioactivity, at six concentrations of ^{32}P in the larval medium. o, 0.05; ●, 0.5; △, 1.0; ▲, 3.0; x, 5.0; *, 10.0, microcuries per millilitre.

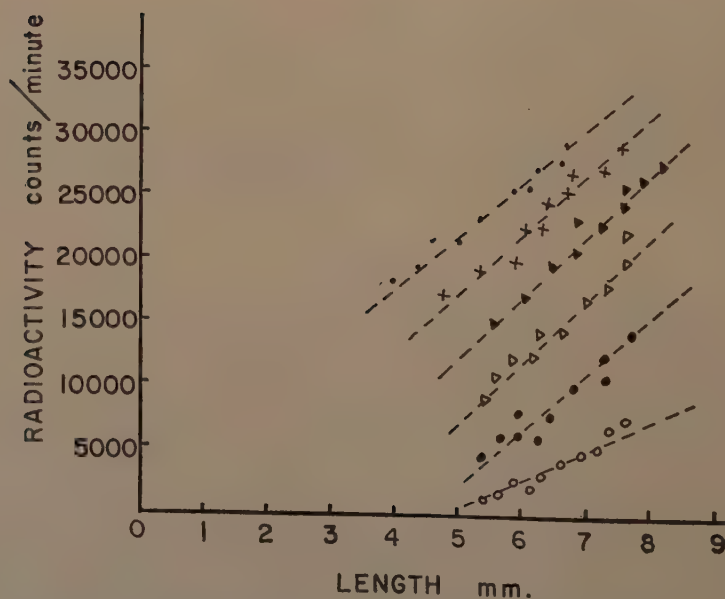


Fig. 2.—Length of larvae of *C. p. molestus* 10 days old, plotted against their radioactivity, at six concentrations of ^{32}P in the larval medium. o, 0.05; ●, 0.5; △, 1.0; ▲, 3.0; x, 5.0; *, 10.0, microcuries per millilitre.

for future measurement of their radioactivity and length. An ocular micrometer in a binocular microscope was used in measuring larval lengths. Emerged adult mosquitos from series II were killed by ether vapour and both their sex and time of emergence were recorded.

The uptake of radio-phosphorus in the larvae was measured by putting each killed, washed larva, after being dried on filter paper, into a counting planchet which was then placed near the window of a Geiger-Muller tube in a counting chamber. The chamber was connected to an 'Echo' automatic scaler. The uptake of radioactivity by the adults killed by ether vapour was measured in the same way. The thickness of the Geiger-Muller tube window was 1.29 mg./cm.², and all samples were measured 1.0 cm. from the window.

All figures for radioactivity reported have been corrected for background and decay of ³²P (half-life 14.3 days). No correction for self-absorption was made in measuring the radioactivity of either larvae or adults because of the thinness of their bodies.

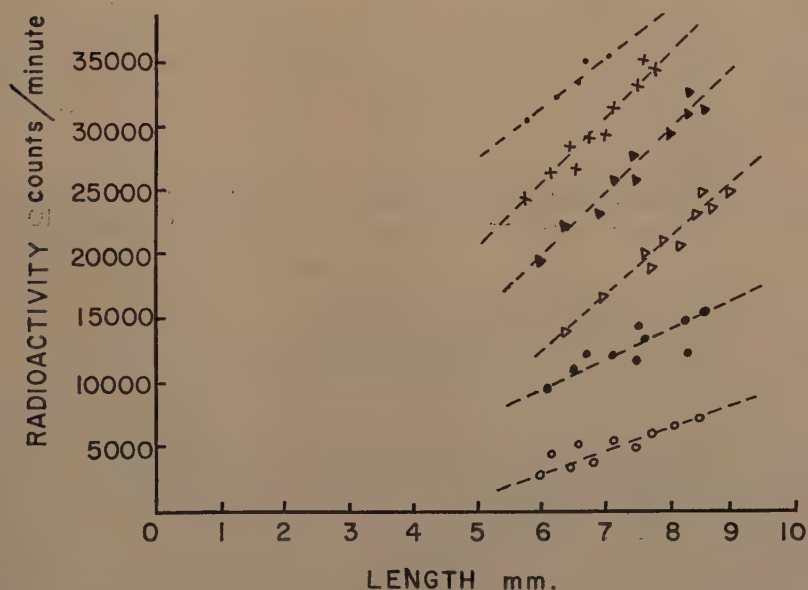


Fig. 3.—Length of larvae of *C. p. molestus* 15 days old, plotted against their radioactivity, at six concentrations of ³²P in the larval medium. o, 0.05; ●, 0.5; △, 1.0; ▲, 3.0; x, 5.0; •, 10.0, microcuries per millilitre.

Experimental results.

Effect of radioactive phosphorus on larval growth and development.

Measurements of larval length and the corresponding radioactivity are recorded graphically in figs. 1 to 4.

It appears that, for all initial concentrations of ³²P in the larval medium, the larger the larva, the greater the uptake of radio-phosphorus.

At an initial concentration of 10.0 microcuries (µc.) of ³²P per ml., about half

of the larvae died within 10 days. After a period of 15 days, only five larvae remained alive. These were then removed and their lengths and radioactivity measured.

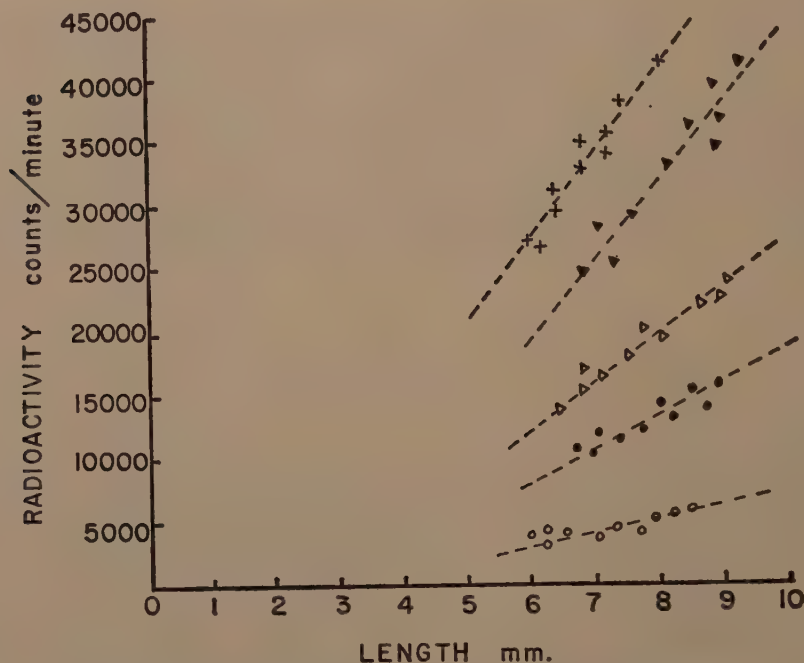


Fig. 4.—Length of larvae of *C. p. molestus* 20 days old, plotted against their radioactivity, at five concentrations of ^{32}P in the larval medium. o, 0.05; ●, 0.5; △, 1.0; ▲, 3.0; x, 5.0, microcuries per millilitre.

The variation of average larval length with time and initial concentration of ^{32}P in the larval medium is recorded in Table II.

From these latter results it appears that radio-phosphorus had little noticeable effect on larval growth up to a concentration of 3.0 $\mu\text{C}/\text{ml}$.; above this concentration, growth was greatly retarded.

TABLE II.

Effect of ^{32}P on growth of larvae of *C. p. molestus*.

Initial conc. of ^{32}P ($\mu\text{C}/\text{ml}$.)	Average larval length in mm., with number of larvae measured in parenthesis			
	5 days	10 days	15 days	20 days
0	5.12 \pm 2.26 (10)	6.77 \pm 2.60 (10)	7.63 \pm 2.76 (10)	7.72 \pm 2.78 (10)
0.05	5.06 \pm 2.25 (10)	6.45 \pm 2.54 (10)	7.11 \pm 2.67 (10)	7.13 \pm 2.67 (10)
0.5	5.17 \pm 2.27 (10)	6.47 \pm 2.55 (10)	7.46 \pm 2.73 (10)	7.81 \pm 2.80 (10)
1.0	5.38 \pm 2.32 (10)	6.49 \pm 2.56 (10)	7.95 \pm 2.82 (10)	7.70 \pm 2.77 (10)
3.0	4.84 \pm 2.20 (10)	6.97 \pm 2.64 (10)	7.46 \pm 2.73 (10)	8.09 \pm 2.84 (10)
5.0	4.93 \pm 2.22 (10)	6.28 \pm 2.51 (10)	6.89 \pm 2.62 (10)	6.82 \pm 2.61 (10)
10.0	4.47 \pm 2.11 (10)	5.45 \pm 2.34 (10)	6.41 \pm 2.53 (5)	—

Effect of radioactive phosphorus on pupation.

Larval development at $17 \pm 2^\circ\text{C}$. and a relative humidity of 75 ± 5 per cent. normally requires about three weeks, but pupation by larvae in media having a concentration of ^{32}P greater than $1.0 \mu\text{c./ml.}$ was prolonged to over five weeks. At concentrations of 3.0 and $5.0 \mu\text{c./ml.}$, only a small percentage of the larvae initially introduced into the media pupated. In concentrations higher than $5.0 \mu\text{c./ml.}$, pupation was completely inhibited, the larvae becoming sluggish, paler in colour and ceasing to feed. They remained at the bottom of the rearing beaker and finally died.

The number of pupae developing in the various concentrations of radio-phosphorus in series II are recorded in Table III.

TABLE III.

Numbers of pupae of *C. p. molestus* formed for various initial concentrations of ^{32}P in larval medium.

Initial conc. of ^{32}P ($\mu\text{c./ml.}$)	0	0.05	0.5	1.0	3.0	5.0	10.0
Number of second-instar larvae introduced	100	100	100	100	100	100	100
Percentage of pupae formed	85	78	75	65	10	3	0

Effect of radioactive phosphorus on adult emergence.

Results of emergence of *C. p. molestus* in all concentrations of ^{32}P used in series II are given in Table IV.

TABLE IV.

Number of adults of *C. p. molestus* emerging from different initial concentrations of ^{32}P .

Initial conc. of ^{32}P ($\mu\text{c./ml.}$)	0			0.05			0.5			1.0			3.0			5.0			10.0		
Number of adults emerging after	♂	♀	Total	♂	♀	Total	♂	♀	Total	♂	♀	Total	♂	♀	Total	♂	♀	Total	♂	♀	Total
25 days	10	19	29	2	4	6	3	4	7	1	2	3	0	0	0	0	0	0	0	0	0
28 days	25	20	44	6	25	31	8	20	28	10	15	25	0	0	0	0	0	0	0	0	0
31 days	1	3	4	2	15	17	2	13	15	6	10	16	0	0	0	0	0	0	0	0	0
34 days	0	2	2	4	10	14	4	10	14	4	2	6	0	1	1	0	0	0	0	0	0
37 days	0	0	0	0	2	2	3	1	4	0	0	0	1	1	2	0	0	0	0	0	0
40 days	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0
Number of larvae introduced	100			100			100			100			100			100			100		
Percentage adult emergence	80			70			68			50			3			0			0		

At concentrations of 5.0 $\mu\text{c.}$ of ^{32}P per ml. or higher, no adults emerged; at 3.0 $\mu\text{c.}/\text{ml.}$ only 3 per cent. emergence was recorded and all the mosquitos died within 48 hours. However, at 1.0 $\mu\text{c.}/\text{ml.}$, the next lower concentration, 50 per cent. of the treated individuals emerged successfully.

Effect of concentration of ^{32}P in the larval medium on the radioactivity of resulting adults.

The amount of ^{32}P in the larval rearing medium determines the amount of radioactivity detectable in the resulting adult mosquitos. The upper limit is fixed by radiation injury which prevents development at higher concentrations of the radioisotope. This is shown in fig. 5, where it can be seen that, in media containing concentrations of ^{32}P greater than 1.0 $\mu\text{c.}/\text{ml.}$, progressively less radioactivity was present in the emerging adults.

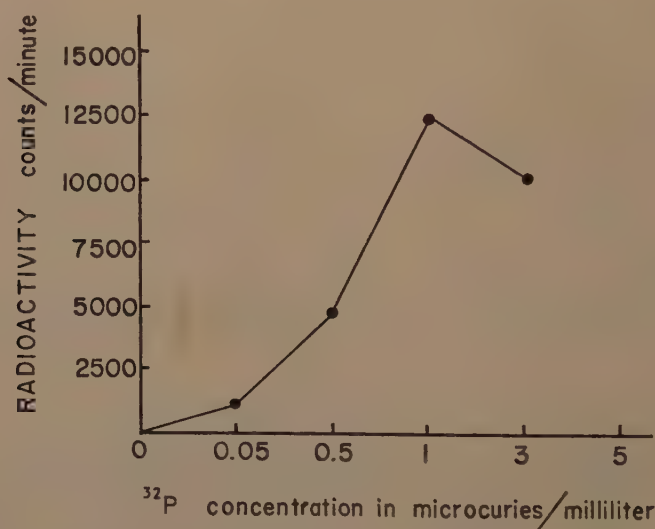


Fig. 5.—Relationship between the amount of radio-phosphorus in adults of *C. p. molestus* and the concentration in the larval medium.

The data given in fig. 5 suggest that, for efficient utilisation of radio-phosphorus in large-scale field experiments, a concentration of ^{32}P of 1.0 $\mu\text{c.}/\text{ml.}$, would be needed, so that emerging adult mosquitos would be sufficiently radioactive to be readily detectable by a scaler or survey meter.

Discussion.

Radio-phosphorus supplied to *C. p. molestus* in the larval medium for the whole of the developmental period obviously does not have an immediate lethal effect on larvae at any of the concentrations used. Above a certain level, the cumulative effect of continued radiation kills older larvae, pupae and adults. The determination of these lethal effects was based on the initial concentration of ^{32}P in the larval medium found necessary to kill individuals prior to emergence of adults. A concentration of 5.0 $\mu\text{c.}$ of ^{32}P per ml. or higher was found to be completely lethal. Most of the deaths occurred in the late larval and pupal stages.

The effect of radiation on growth is dependent on the dosage. At low dosages

there is little effect; at intermediate dosages there appears to be enhancement in some cases and retardation in others; at high dosages, growth may be inhibited or retarded, but in the latter case it nevertheless continues for a long period.

The effect of radiation on development is also dependent on the concentration of ^{32}P in the larval medium. At concentrations up to and including $1.0\text{ }\mu\text{c./ml.}$, pupation occurred as normally. In media with concentrations higher than 1.0 , but less than $5.0\text{ }\mu\text{c./ml.}$, pupation occurred two weeks later than in the controls; but in concentrations over $5.0\text{ }\mu\text{c./ml.}$ pupation was completely inhibited and larvae eventually died.

Hassett & Jenkins (1951) found that larvae of *A. aegypti* reared in concentrations of $0.5\text{ }\mu\text{c.}$ of ^{32}P per ml. were slightly retarded; at $5.0\text{ }\mu\text{c./ml.}$, growth was largely inhibited and pupation delayed four weeks. At concentrations of $10\text{--}25\text{ }\mu\text{c.}$ of ^{32}P per ml., larvae became pale, did not feed and died. Arnason, Irwin & Spinks (1949) also found that higher concentrations of ^{32}P in the medium affected the growth, pupation and adult emergence of fruit-flies. Halberstaedter, Goldhaber & Hecht (1943), in their study of the effect of X-rays on development of insects, found that blowfly larvae X-irradiated with $4,000\text{--}5,000\text{ r}$ did not develop into adults; Whiting (1950) reported that mature larvae of the Mediterranean flour moth, *Anagasta kuhniella* (Zell.), X-irradiated with $40,000\text{--}160,000\text{ r}$ resembled diapause larvae, showing an arrest of growth.

It is probable, therefore, that radiation effects on growth and development include disruption of enzyme systems and the inhibition of various syntheses necessary for pupal differentiation. When pupation fails to occur as a result of irradiation, one of the possible mechanisms causing this may be an adverse effect of the radiation on the ring gland whose hormone is known to influence pupation (Hadorn, 1937, and others).

Summary.

In a study of the effect of different concentrations of radioactive phosphorus (^{32}P) in the larval medium on the growth and development of *Culex pipiens molestus* Forsk., ^{32}P was found to have little noticeable effect on the growth of the larvae up to a concentration of 3.0 microcuries ($\mu\text{c.}$) of ^{32}P per ml., but, above this concentration, larval growth was greatly retarded.

The period of larval development was increased at concentrations greater than $1.0\text{ }\mu\text{c.}$ of ^{32}P per ml., and pupation occurred two weeks later than in the controls. In concentrations higher than $5.0\text{ }\mu\text{c./ml.}$, pupation was completely inhibited, larvae became sluggish, stopped feeding and finally died.

The effect of ^{32}P in the larval medium on the emergence and radioactivity of the resulting adults was also studied. On the basis of this study, it is recommended that, for efficient utilisation of radio-phosphorus in large-scale field experiments, a concentration of ^{32}P of $1.0\text{ }\mu\text{c./ml.}$ be employed so that emerging adult mosquitos may be sufficiently radioactive to be readily detectable.

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OBSERVATIONS ON THE HABITS OF MOSQUITOS OF PLATEAU
PROVINCE, NORTHERN NIGERIA, WITH PARTICULAR
REFERENCE TO *Aedes (Stegomyia) vittatus*
(BIGOT).

By J. P. T. BOORMAN, M.A.

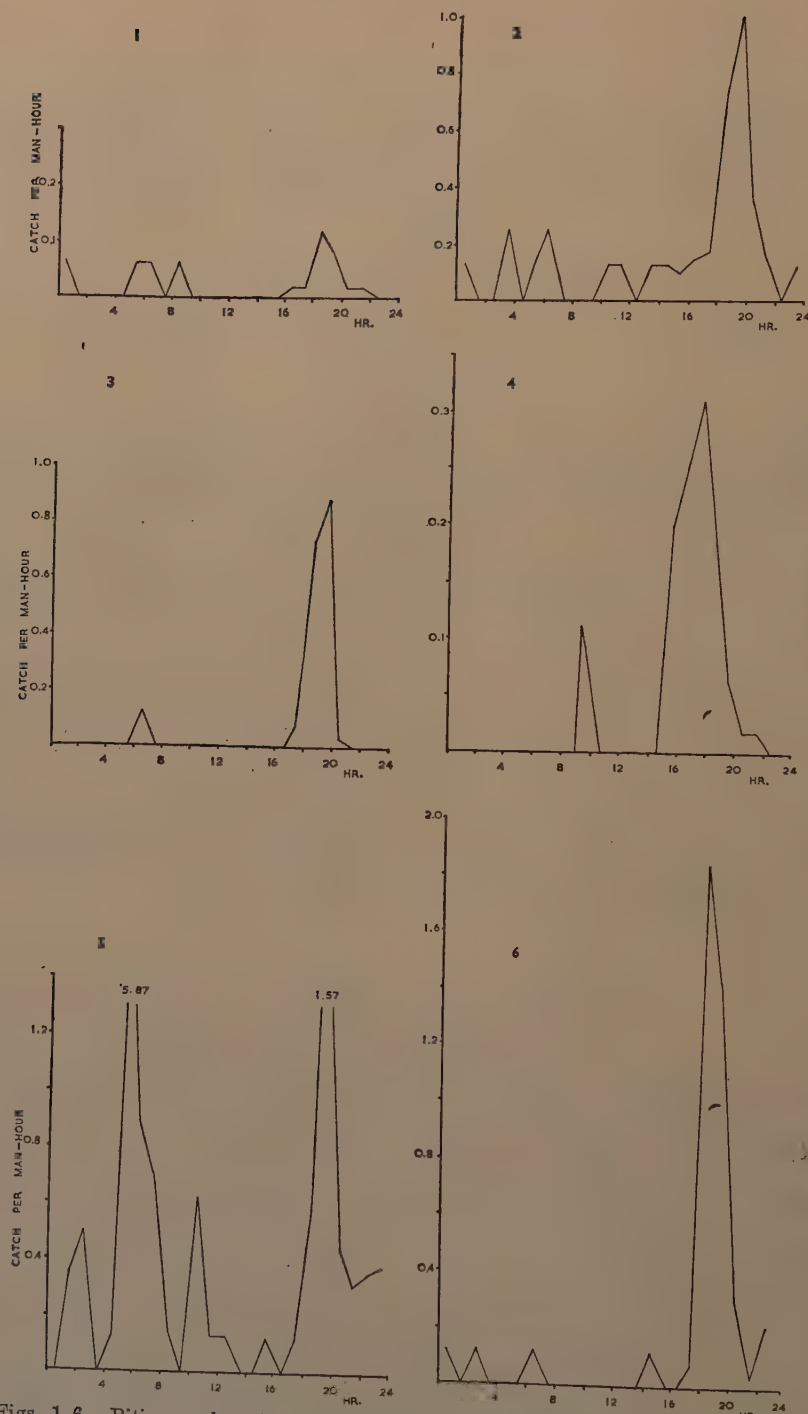
*Virus Research Unit, West African Council for Medical Research,
Yaba, Lagos, Nigeria.*

From time to time, outbreaks of jaundice of unknown aetiology have occurred in Northern Nigeria, especially in the Jos area of Plateau Province. Such was the case during the years 1950-51, when numerous cases of jaundice with several deaths were reported. This outbreak was shown not to be due to yellow-fever virus (Anon., 1952), but 15 of 87 schoolchildren from the Jos area tested for the presence of yellow-fever antibodies in 1951 had changed from negative to positive when re-tested a year later (Anon., 1954). Another epidemic, clinically somewhat similar in nature to the earlier one, but with fewer cases, occurred in the same area early in 1960. While the exact nature of the disease is unknown, the possibility of an epidemic of an arthropod-borne virus disease cannot be ruled out.

It was with this in mind that the present study was carried out, to investigate the breeding and biting habits of those mosquitos most likely to be involved in virus transmission. Little information concerning the mosquitos of Plateau Province is available; various reports, however, have indicated that one of the commonest mosquitos in the area is *Aedes (Stegomyia) vittatus* (Big.). Particular attention was paid to this species since it has been shown to be an efficient vector of yellow-fever virus in the laboratory (Philip, 1929), and has been suspected as a vector in an epidemic of yellow fever in the Sudan (Kirk, 1941). The investigations were carried out during the period April to June 1960.

The site of the investigations.

Most of the work described below was carried out at Vom and Jos. The Jos Plateau area lies roughly between 10° 30' and 09° 00' N., and 09° 30' and 08° 30' E. It is mostly over 3,000 ft. above sea-level, rising in some places to well over 5,000 ft. It is drained by a number of rivers, those to the east, west and south draining into the Niger and Benue rivers, and those to the north to Lake Chad. It is rich in minerals; tin, columbite and tantalite are mined extensively from open-cast workings. There are numerous rocky outcrops of granite separated by flat areas of grassland; during the rains, water collects in depressions in exposed rock and forms breeding sites for mosquitos. The people belong to a number of tribes, the most numerous being the Hausas, Fulani and Birom; cattle-farming is carried on extensively. The Fulani are mainly a nomadic people and live in small, portable huts made of a wooden framework covered with grass. Many of the Birom villages are surrounded by dense hedges of a prickly cactus-like *Euphorbia*. The huts are of mud, with roofs of thatch or corrugated iron; the villages are generally very clean, and there is a lack of potential mosquito breeding places in the form of empty tins and bottles. Annual mean temperatures range from 70 to 75°F.; rainfall over the area is between 40 and 60 in. It is not unusual for temperatures to fall as low as 40-50°F., especially at night during the dry season; very occasionally, ground temperatures drop below freezing point. A



Figs. 1-6.—Biting cycles of the six most abundant species. (1) *Aedes ingrami*; (2) *A. vittatus*; (3) *A. africanus*; (4) *A. aegypti*; (5) *Culex univittatus*; (6) *A. luteocephalus*.

violent hailstorm occurred at Jos during one of the mosquito catches of the present survey.

The rainy season extends usually from May to October. During the rest of the year, the country is very dry; the humidity falls to a low level and for much of the dry season a warm dry wind or 'harmattan' blows from the north. Most of the water supplies of the villages come from deep wells, or, during the rains, from streams. Water is not stored in pots, and during the dry season almost all potential mosquito breeding sites are dry.

The species encountered.

A list of the species encountered, with the names in full, the localities and stages in which they were found, is given below. Only those species determined to specific level have been included.

- Anopheles (Myzomyia) gambiae* Giles: larvae and adults, Vom and Jos.
- Toxorhynchites erythrurus* (Edw.): adults, Jos.
- Uranotaenia ornata* Theo.: larvae, Vom and Jos.
- Ficalbia (Mimomyia) plumosa* (Theo.): adults, Jos and Vom.
- ✓ *Aedes (Finlaya) ingrami* Edw.: larvae, adults, Jos.
- ✓ *A. (Stegomyia) aegypti* (L.): larvae and adults, Jos and Vom.
- A. (S.) simpsoni* (Theo.): larvae and adults, Vom and Jos.
- ✓ *A. (S.) africanus* (Theo.): adults, Jos and Vom.
- ✓ *A. (S.) luteocephalus* (Newst.): larvae, Jos and Vom, adults, Jos and Vom.
- ✓ *A. (S.) vittatus* (Big.): larvae and adults, Jos and Vom.
- Culex (Lutzia) tigripes* Grp.: larvae, Jos and Vom.
- C. (Culiciomyia) nebulosus* Theo.: larvae, Vom.
- C. (Mochthogenes) inconspicuus* (Theo.): larvae, Vom.
- ✓ *C. (Culex) univittatus* Theo.: adults, Jos and Vom.
- C. (C.) decens* Theo.: larvae, Jos and Vom.

Catches of adult mosquitos.

Catches using human bait.

Nine catches were carried out at various locations in the Jos area; in each case they were in places where active breeding of *A. vittatus* had been found to occur but were not less than two hundred yards from the nearest village. The terrain in all cases was rocky and the catchers sat partly sheltered by large rocks or small trees. In most cases, only one catching station was used; this was manned by two catchers throughout the period of the catch. The total number of man-hours worked was 341. The species taken were *Ficalbia plumosa*, 1 ♀; *A. ingrami*, 23 ♀; *A. aegypti*, 1 ♂, 38 ♀; *A. simpsoni*, 4 ♀; *A. africanus*, 1 ♂, 68 ♀; *A. luteocephalus*, 148 ♀; *A. vittatus*, 10 ♂, 102 ♀; *A. (Aëdimorphus)* sp. indet., 3 ♀; *Eretmapodites* sp. of the group of *E. chrysogaster* Grah., 3 ♀; *Culex univittatus*, 5 ♂, 169 ♀; *Anopheles* sp. indet. (three species) 24 ♀. The hourly catches of *Aedes aegypti*, *A. ingrami*, *A. africanus*, *A. luteocephalus*, *A. vittatus* and *C. univittatus* are presented in figs. 1 to 6.

Hand-catches.

Four hand-catches, each of half an hour's duration, were carried out at Jos near the site of the baited catches. In these, the vegetation was disturbed with a stick, and any mosquitos seen to fly up were caught in a small net. These catches yielded *Ficalbia plumosa*, 1 ♂; *Toxorhynchites erythrurus*, 1 ♀; *A. aegypti*, 4 ♂, 4 ♀; *A. simpsoni*, 2 ♀; *A. ingrami*, 1 ♀; *A. africanus*, 1 ♂; *A. luteocephalus*, 1 ♀; *A. vittatus*, 8 ♂, 6 ♀; *Eretmapodites* sp. *chrysogaster* group, 1 ♂; *C. univittatus*, 10 ♂, 11 ♀. None of the female mosquitos taken was blood-fed.

Spray-catches.

Two spray-catches were carried out at a group of five huts at least a mile from the nearest village and about 200 yd. from the site of the rock-hole survey described below. The spray used was pyrethrum extract, 1:100 in kerosene. In the first catch, one hut used as a kitchen and sprayed between 1800 and 1830 hr. yielded 15 ♀ of *Anopheles* sp. and 3 ♂, 4 ♀ of *Culex* sp. A second hut, used for sleeping, yielded 1 ♂, 68 ♀ of *Anopheles* sp. only. In the second catch, from 0530 to 0630 hr., the results were: hut 1, in which six adults slept, 2 ♀ of *Culex* sp. and 5 ♂, 115 ♀ of *Anopheles* sp.; hut 2, in which two adults had sheltered from 0100 to 0400 hr., 6 ♂, 24 ♀ of *Anopheles* sp.; hut 3, which was unoccupied, 7 ♂ and 3 ♀ of *Anopheles* sp.

Studies of mosquito breeding sites.

A number of short surveys for mosquito breeding sites were carried out, all at Vom. The species found, with the situations in which they were found, were:—*A. gambiae*, one larva, bamboo pot; *U. ornata*, in plant axils; *Aedes ingrami*, in tree holes; *A. aegypti*, in tree holes, bamboo pots, sisal leaves, one record from a rock hole; *A. simpsoni*, plant axils; *A. luteocephalus*, tree holes, bamboo pots; *A. vittatus*, rock holes; *C. nebulosus*, bamboo pots; *C. inconspicuus*, stream, among vegetation; *C. decens*, water in petrol drum, rock hole.

It was noticed at Vom that, after heavy showers of rain, water tended to collect in the horizontal leaves of sisal plants. The amount of water varied from a few drops to about 20 ml. Of 11 such leaves which contained water, two contained larvae of *A. aegypti*. These larvae were in the second instar; even the larger collections of water had dried out in three days, and it is doubtful if such larvae would have been able to complete their development.

A survey of larvae inhabiting the axils of wild banana plants (*Musa* sp.) yielded two species only: *A. simpsoni* and *U. ornata*. Of 58 axils examined, 38 contained larvae, usually of both species.

As it is well known that the normal breeding sites of *A. vittatus* are rock holes, two surveys of mosquitos breeding in this type of habitat were carried out. In the first, in the Jos area, rock holes, which had filled with heavy rain the night previously, were classified as either 'shallow' (less than one inch deep) or 'deep' (more than one inch deep). Of 30 holes examined, 19 were of the shallow type, the other 11 varied from one inch to one foot in depth. Of the shallow holes, 18 contained clear water but no larvae. The remaining one was muddy and contained larvae of *A. vittatus*. Of the deep holes, five contained muddy water and one of these had larvae of *A. vittatus*. All of the remaining six holes, in which the water was clear, contained larvae of *A. vittatus*. No other mosquito species was present. At noon, the temperatures of nine rock holes fully exposed to bright sun were: shallow holes, 34.0, 34.6, 35.8 and 31.4°C., deep holes, 28.6, 31.4, 29.8, 31.2 and 33.2°C.

The second rock-hole survey was carried out at Vom over a period of three weeks, when the temperature and rainfall records were as shown in Table I; a number of rock holes or depressions in the rock surface were numbered and examined each day for the presence of larvae. All larvae found were presumed to be *A. vittatus*, except those that were obviously *Culex*; in fact, examination of samples of larvae from many holes revealed no other species except *C. tigripes*, *C. decens* (once) and *A. aegypti* (once). The site selected for the survey was a large granite outcrop about 300 ft. high, forming the end member of a chain of similar outcrops and surrounded by flat swamp and grassland. Apart from the five huts mentioned in the section on spray-catching that were about 200 yd. from the survey site, the nearest human habitations were about a mile distant.

When first examined, on 24th May, all the rock holes were dry, and about 70 rock holes were numbered. Some of the holes contained a little dust, others dry

mud and leaves. On this day, holes 32 to 37, 56 to 61, and 64 and 68 were filled with tap-water. In hole 60, a deep-type hole, active Chironomid larvae were observed an hour after filling with water, although the hole had previously contained only dry mud. These larvae were not identified, but it was possible that they were *Polypedilum vanderplanki* Hinton (Hinton, 1951, 1960), a species that has been shown to be resistant to desiccation in the larval stage.

TABLE I.

Maximum and minimum temperatures and rainfall at Vom during the period of the rock-hole survey.

Date	Temp. (°F.)		Rainfall (in.)
	Max.	Min.	
May			
15	79	66	0.16
16	82	64	0.47
17	78	64	0.89
18	78	60	0
19	82	62	0
20	82	65	0
21	85	66	0
22	85	64	0
23	86	66	0
24	82	66	0
25	82	65	0.03
26	81	65	0.175
27	80	65	0.105
28	78	63	0.49
29	80	64	1.51
30	78	66	trace
31	82	64	0.005
June			
1	84	62	0
2	82	63	0.11
3	80	64	0.88
4	78	63	0.62
5	77	62	0
6	82	67	0
7	81	66	0.08
8	78	65	0.68
9	78	61	0
10	80	64	0
11	80	67	0
12	78	67	0.81
13	79	62	0.005
14	76	65	0.67
15	78	62	0

Although a trace of rain fell during that night, by the next morning all the holes were again dry except 68, which contained wet mud and a few dragonfly nymphs. All the holes mentioned above were refilled with tap-water. That night, more rain fell, and by the next morning (26th May) all the holes contained water. Thereafter, some rain fell on most days, and many of the holes contained water throughout the survey.

The observations made on 30 of the holes are summarised in Table II. Records of the various instars of *A. vittatus* larvae are denoted by 1, 2, 3, 4 or P (denoting pupa). Dry holes were recorded as D, damp mud (when the surface was visibly

TABLE II.
Daily observations of 30 rock holes at Vom.

Day	Rock-hole number																															
	1	2	3	4	6	8	9	14	15	16	18	24	25	26	29	30	32	33	34	36	38	40	41	42	44	58	67	68	69	70		
May																																
26																																
27	1	1	1	1	1		1	1	1	1	1	1	1	1	2	12	123	123	123	123	1	D	D			DM	23	123	123	123	123	123
28	2	2	2	2	23		23	12	12	2	2					1234	234	234	1234	1234	12					23	1234	1234	1234	1234	1234	1234
29	4	123	123	1234	WM	WM	234	23	23	23	23					1234	1234P	1234P	1234P	234P	12					24	D	D	D	234	234	1234
30	4	23	23	234	34	8	23	23	23	23	23	23	23	23	23	234P	1234P	1234P	234P	234P	123					1234	D	D	D	1234P	1234P	1234
31	WM	1234	1234	1234P	WM	WM	34P	23	1234		1234	D	8	4	234P	234P	1234P	1234P	234P	1234P	1234					1234	D	D	12	1	1234P	1234P
June																																
1	D	1234P	1234P	1234P	D	D	WM	WM	3	WM	WM	D		4P	4P	234P	234P	1234P	1234P	1234P	123					123	23	2	1234P	1234P	1234P	1234P
5	12	234P	1234P	1234P	12	D	2	1234P	1234	2	2	D	2	12P	2	1234P	1234P	1234P	1234P	123P	12	1234P	1234P	1234P	1234P	123	12	234P	234P	1234P	1234P	1234P
6	WM	1234P	1234P	1234P	23	WM	2	234P	1234	2	WM	D	3	3	23	23	1234P	23	1234P	234P	123	123	1234P	1234P	1234P	1234P	123	1234P	1234P	1234P	1234P	1234P
7	WM	1234P	1234P	1234P	D	D	D	DM	DM	DM	DM	D	D	84	D	1234P	WM	234P	D	123	23	1234P	1234P	1234P	1234P	WM	2	D	D	1234P	1234P	1234P
8	2	1234P	1234P	1234P	1234P	12	1	1	1	1	1	1	1	1234	2	1234P	234	234P	2	123	1234	1234P	1234P	1234P	1234	1	1	2	1234P	1234	1234	1234
9	12	234P	234P	234P	234P	12	12	12	2	2	2	2	2	34	2	1234P	1234	234P	2	123	1234	1234P	1234P	23P	1234P	12	2	12	1234P	1234	1234	1234
10	12	234P	1234P	1234P	234	2	2	12	D	12	23	12	23	134P	1	234	134	34P	123	123	1234P	1234	1234	23P	1234P	12	2	12	1234P	1234	1234	1234
11	DM	34P	1234P	1234P	WM	WM	WM	WM	D	3	WM	D	34	124P	123	1234P	234P	24P	18	123	1234P	1234P	1234P	234P	1234P	123	123	1234	1234P	1234P	1234P	1234P
12	1	234P	1234P	1234P	1234P		1	1	1	13			134	124P	1234	234P	1234P	34P	1234	123	1234P	1234P		234P	1234P	1234	1234	1234	1234P	1234P	1234P	1234P
13	12	1234P	1234P	1234P	1234P	1	12	12	2	12	234	12	14	1234P	1234	1234P	234P	4	234	123	1234P	1234P		234P	1234P	1234	1234	1234	1234P	1234P	1234P	1234P
14	23	124P	1234P	1234P	234	12	23	12	123	234	234P	12	124P	124P	234	124	234	4	234	123	1234P	234	234		234P	234	1234	1234	1234P	1234P	1234P	1234P
15	23	84P	23	1234P	234	WM	234	12	12	3	234	23	234P	1234P	24P	124	234	1	1234P	123	1234P	1234P	1234P		234P	1234	1234P	1234P	1234P	1234P	1234P	1234P
16							WM	WM	3	D	DM	D	D	1234P	1234P	2	1234P	1	1234P	1234	1234P	1234P	24		234P	1234P	1234P	1234P	1234P	1234P	1234P	1234P

1, 2, 3 and 4 against the dates denote larval instars; P, pupae; D, hole dry; DM, damp mud only; WM, wet mud only; a blank indicates no observation on that day.

damp but there was no free water) by DM, and wet mud (mud with a trace of free water on the surface) by WM. Brief descriptions of the holes mentioned in the table are:

1. Circular, 7" diam. \times 2½"; 2" mud and some grass. Water clear.
2. Oval, 24 \times 30 \times 11"; 1" mud. Water turbid.
- 3, 4. Circular, 15" diam. \times 7"; ½" mud. Water turbid.
- 6, 8, 9. Oval, 15 \times 7 \times 4"; 3" rotting stems and seeds. Water clear.
- 14, 15, 18. As above, but no rotting stems or seeds.
16. As nos. 6, 8, 9, but with a thin bacterial scum on the surface.
- 24, 25, 26. Circular, 5" diam. \times 3".
29. Circular, 11" diam. \times 1½"; ½" mud.
30. Circular, 21" diam. \times 2". Water clear, with 1" mud and plants growing in the water.
- 32, 33. Oval, 8 \times 14 \times 3½"; water clear, with 1½" mud, rotting leaves, dragonfly nymphs and tadpoles.
- 34, 36. As above, but only ½" mud.
38. Oval, 30 \times 18 \times 1½"; water clear, with a little mud only.
- 40, 41. Circular, 15" diam. \times 3". Water clear, with mud, rotting leaves, dragonfly nymphs and tadpoles.
42. Circular, 15" diam. \times 3". Shaded, water clear, almost full of mud and rotting plant debris. Many nematodes, Chironomid larvae, Ceratopogonid larvae, dragonfly nymphs and tadpoles.
44. Circular, 18" diam. \times 6". Water clear, ½" mud, with dragonfly nymphs and tadpoles.
58. Circular, 6" diam. \times 4". Water clear with 2" mud.
67. Circular, 16" diam. \times 5". Water clear, little mud and two dead leaves.
68. Oval, 21 \times 12 \times 2"; water clear, with a little mud and dead leaves.
- 69, 70. Oval, 15 \times 7 \times 4"; water clear, 1" mud.

Nineteen of the total number of holes studied either remained dry throughout the period of the survey, or contained water briefly but never larvae. Twelve of these were merely shallow depressions in the rock surface, less than one in. deep, and varying from three to 36 in. in diameter. The other seven were less than three in. deep and less than six in. in diameter; it was possible that some fault in the rock allowed the water to drain away from these holes. Seven other holes contained larvae on only one or two occasions, and then only in the first or second instar. One of these, which was always full of water, was very large, being three ft. across and two ft. deep. Larvae were recorded once from this hole; they were in the first instar. Egg-rafts of *Culex* species were seen on two occasions, but no larvae. A large population of predators—dragonfly nymphs, beetles and tadpoles—was present, and the water was usually foul. Another hole, 18 in. across and six in. deep, also always contained water, but the surface was covered with a thick bacterial scum. Although numerous dragonfly nymphs and tadpoles were present, no mosquito larvae were seen. The total number of holes studied was 76; the records of the 30 detailed in Table II are representative of the rest of the holes.

As mentioned above, egg-rafts of *Culex* species were found in holes in association with larvae of *A. vittatus* on one or two occasions. Larvae of *C. tigripes* were found on several occasions; it is possible that the egg-rafts belonged to that species. The dragonfly nymphs were of unidentified species in the Anisoptera; they were found exclusively in those holes containing mud and decaying vegetable matter. Numerous species of water beetles were seen; several Corixid species (Hemiptera) were recorded on odd occasions and these and the beetles had presumably flown into the pools when they filled with water, and were temporary residents. A water scorpion (NEPIDAE, Hemiptera) was also seen on one occasion. All of these presumably preyed upon mosquito larvae; it was noticeable that larvae were

seldom recorded in large numbers from holes where many predators were present, although the presence of predators did not prevent larvae from reaching maturity. Those holes which had much mud also supported a population of Ceratopogonid and Chironomid larvae. The tadpoles belonged to an unidentified species of small greenish-brown frog; the eggs were laid in small batches of about 20 or 30 eggs each in both large and small holes. The tadpoles were small, about half an inch long during the survey, and black in colour; they had powerful jaws armed with sharp, black teeth. Tadpoles were observed attacking and devouring mosquito larvae on several occasions, and the mid-guts of several tadpoles dissected in the laboratory all contained fragments of mosquito larvae. On one occasion a complete larval siphon, readily identifiable as belonging to a fourth-instar larva of *A. vittatus*, was found in a tadpole gut.

In order to form some estimate of the numbers of adult mosquitos produced from the rock holes, pupae were removed from 18 of the holes daily and counted. Some holes produced many more larvae than others, for no apparent reason save that they were larger in size. Thus, hole 2 produced 920 pupae in nine days, the maximum in one day being just over 300. During the same period, 189 pupae were removed from hole 3. From other holes the maximum number of pupae removed in any one day was 78. In all, 89 daily collections from 18 holes yielded 2,696 pupae; a mean of approximately 30 pupae per hole per day.

The temperature of the water in two typical holes, 69 and 70, measured on a clear sunny day at noon, was 31.8 and 31.6°C., respectively.

Laboratory studies.

All of the laboratory studies were performed on larvae or pupae of *A. vittatus*.

The length of the pupal stage.

Pupae of an age known to within four hours were placed in 3" × 1" glass tubes and allowed to hatch in the laboratory. Ten pupae hatched in periods ranging from 24 to 46 hours.

The ability of pupae to hatch in the absence of free water.

Wild-caught pupae were washed gently in clean water and transferred to damp filter paper in 3" × 1" glass tubes. Of 11 pupae so treated, all produced adults within 48 hours.

The sex ratio in wild pupae.

Wild-caught pupae, taken at random from rock holes, were washed in clean water and allowed to emerge into cages in the laboratory. The numbers of each sex emerging were counted, no allowance being made for the small number that failed to hatch. Three trials gave the following results: males, 40, 92, 37, respectively (total 169), females, 92, 46, 42, respectively (total 180). The ratio of males to females was 1:1.1.

The ability of pupae to withstand drying.

Wild-caught pupae were washed gently in clean water and filtered on to glass wool; they were transferred with a needle to dry filter paper to remove surface water and placed on dry filter paper in 600-ml. beakers for varying periods. At the end of each experimental period, 300 ml. water was added to each beaker. Surviving pupae were counted 12 hours later. Two control groups were similarly treated but re-wetted immediately after drying. The results are shown in Table III.

The ability of larvae to withstand drying.

The method used was similar to that used for pupae, and the results are detailed in Table III.

TABLE III.

Resistance of pupae and larvae of *A. vittatus* to desiccation.

Period of drying (hr.)				No. surviving/ No. used	Percentage surviving
				<u>A: pupae</u>	
$\frac{1}{2}$	30/30	100
1	34/34	100
$1\frac{1}{2}$	29/34	85.4
2	24/25	96
6	0/27	0
Control	33/33	100
Control	35/35	100
				<u>B: larvae</u>	
$\frac{3}{4}$	36/38	95
$1\frac{1}{4}$	29/32	91
Control	28/28	100

The ability of larvae and pupae to withstand high temperatures.

Wild-caught fourth-instar larvae and pupae were washed in clean water and transferred to a thin-walled 3" x 1" aluminium cylinder, one end of which was open and the other closed with fine-mesh nylon gauze held in place with a rubber band. The cylinder, with batches of larvae or pupae, was drained quickly of excess water and lowered into a bath of water at the desired temperature. At the end of exposure, the cylinder was drained rapidly and lowered into water at room temperature. Larvae or pupae were pipetted gently into 600-ml. beakers and the numbers surviving after 12 hours noted. Controls were performed in the same manner but the hot bath was omitted. The results are shown in Table IV.

Survival of dried eggs and larvae.

Four samples of scrapings from dry rock holes from an area at Jos, where *A. vittatus* was known to occur, were soaked in water. No larvae hatched from these samples, which were taken from 20 rock holes, on 20th May, a few days after heavy rain had fallen.

Two samples of damp mud, from holes at Vom which had contained water and active larvae the previous day, were placed in a beaker of water. From one sample, no larvae were recovered; from the other, living first- and second-instar larvae were observed ten minutes after soaking.

Batches of eggs laid by caged females at Vom were dried at air temperature on the filter paper on which they were laid and stored in an airtight jar in the laboratory at 73°F. One batch, re-soaked ten weeks after laying, hatched successfully; another, re-soaked after 18 weeks, failed to hatch.

Attempts to found a colony of A. vittatus.

Wild larvae and pupae from Vom were placed in 250-ml. beakers in an 18-in. cube cage of mosquito netting. Emerging adults were provided with a petri dish containing 10 per cent. glucose solution, and given the opportunity to bite a

TABLE IV.

The ability of larvae and pupae of *A. vittatus* to withstand high temperatures.

Period of exposure	Temperature (°C.)	Instar	No. surviving/ No. tested	Percentage surviving
Control	36.5	L	12/12	100
Control		P	12/12	100
15 min.		L	23/24	96
1 hr.		L	25/25	100
15 min.		P	25/25	100
1 hr.		P	26/26	100
1 min.	45.5	L	14/14	100
3 min.		L	11/13	84
5 min.		L	25/30	83
1 min.		P	16/18	89
3 min.		P	1/19	5
5 min.		P	1/20	5
1 min.	48.0	L	11/12	92
3 min.		L	1/14	7
1 min.		P	12/12	100
3 min.		P	0/14	0
$\frac{1}{2}$ min.		L	0/13	0
1 min.		L	0/21	0
$\frac{1}{2}$ min.	52.5	P	4/19	21
1 min.		P	0/19	0
15 sec.		L	0/16	0
15 sec.		P	0/23	0

TABLE V.

Locality records of *A. vittatus* in Nigeria.

Locality	N. lat.	E. long.	Altitude (ft.)	Rainfall (in.)	Veg. zone
Abeokuta (M2)	07.09	03.21	500	40	GS
Bamenda (S)	05.56	10.10	5800	103	GS/M
Baro (M2)	08.35	06.18	500	55	GS
Ekpoma (S)	06.45	06.08	500	50	GS
Enugu (M1)	06.27	07.29	700	70	GS
Funtua (M2)	11.32	07.15	2500	55	SS
Gadua (M2)	11.50	10.12	1000	30	SS
Ibadan (M2)	07.23	03.50	700	50	RF
Ibi (M2)	08.11	09.43	500	45	GS
Idanre (B)	07.07	05.06	1700	48	RF
Ikoyi (Lagos) (M2) ..	06.20	03.20	500	70	RF
Jos (B)	09.55	08.53	4500	56	GS
Kaduna (M2, B, S) ..	10.30	07.28	1900	45	GS
Kakuri (M2)	10.26	07.26	1500	45	GS
Kano (M2)	12.02	08.32	1500	34	SS
Katagum (M2)	12.18	10.20	1000	20	SS
Katsina (M2)	13.01	07.30	1800	35	SS
Kumba (S)	04.38	09.25	800	91	RF
Lokoja (M2)	07.48	06.44	500	50	GS
Ogbomosho (M2)	08.06	04.12	1100	40	GS
Okene (E)	07.34	06.13	1200	50	GS
Oshogbo (M2)	07.47	04.29	1000	45	GS
Shaki (M2)	08.34	03.19	1500	40	GS
Vom (B)	09.43	08.47	3400	56	GS
Yola (M2)	09.13	12.29	900	40	SS
Zungeru (M2)	09.45	06.05	600	45	GS

These localities are plotted on the map (fig. 7).

guineapig and a rat (both anaesthetised with nembutal) each day. It was noted that the mosquitos fed more readily when the animals were placed on top of the cage, rather than inside. Dishes containing vertical strips of filter paper held on wire frames and with the lower end of the paper dipping into one inch of water in a 250-ml. beaker were provided for egg-laying; a small piece of rock with a water-filled depression on top was also provided. Despite the frequent introduction of fresh stocks of larvae and pupae, the mortality was high, whether the cage was kept indoors or out, shaded or unshaded. A few eggs were laid on the damp filter paper, but the rock appeared to be ignored. In the laboratory, most eggs were laid on the side of the filter paper that received the most light, *i.e.*, that facing the windows. Mating was not observed, although this presumably occurred since at least some of the eggs were fertile. It was noted that the time of development of wild-caught larvae kept in the laboratory was longer than that observed for larvae in their natural habitat.



Fig. 7.—Sketch map showing relation of distribution of *A. vittatus* in Nigeria to the rainfall in inches.

The distribution of *Aedes vittatus* in Nigeria.

The records of *A. vittatus* from Nigeria which are known to the author are listed in Table V. They are culled from various reports, papers and collections; those of the author are denoted by (B), those of Mr. Elliott and Mr. Service (formerly of the Federal Malaria Service, Ministry of Health, Lagos) by (E) and (S), respectively, of Mattingly (1947) by M1 and of Mattingly (1952) by M2. GS denotes Guinea Savannah; SS, Sudan Savannah; RF, rain-forest; and M, montane.

Discussion.

Behaviour of the adult mosquitos.

The small number of mosquitos taken, 585, compared with the total man-hours worked, 341, is not surprising considering that the catches were carried out at the end of the dry season, before heavy rain. This being so, it is interesting and perhaps significant that tree-hole and rock-hole breeding species were present and found biting in relatively large numbers. The search for tree holes containing water was largely unsuccessful, particularly in the Jos area; but it seems hardly likely that such large numbers of adults should have survived the dry season. A more likely explanation is that there exist, through most of the dry season, foci of breeding which are continually producing small numbers of adults. These foci could be very deep tree holes or concealed and sheltered deep rock holes, in which some water remains throughout the dry season and from which rapid evaporation is restricted by the fact that they are hidden. This explanation does not, of course, hold good for *C. univittatus*, which was taken in large numbers; the typical breeding places of this species are stagnant pools, marshes, etc. However, breeding places for this species in the form of marshes and pools at the edges of some of the streams that flow throughout the year could account for the presence of adults. Almost all of the mosquitos taken appeared to be fresh, and were not very worn as would be expected with individuals that had been on the wing for a long time.

The results of the baited catches are expressed as the catch per man hour. The various methods of treating the results of baited catches are discussed by Haddow (1954), who points out that the most satisfactory method is to plot the geometric mean catch against time. In the present series of catches, however, the catches were of varying duration, some being over the full 24-hour period while others were of four to eight hours' duration only. It was therefore considered preferable to express the results as catch per man-hour, rather than as geometric mean catch per hour.

Of the *Stegomyia* species, the most numerous was *A. luteocephalus*. This species has been shown to be a vector of yellow-fever virus in the laboratory (Bauer, 1928), and it would be surprising if it were not found to be a vector of some of the closely related members of the arthropod-borne virus group. It has been suspected as a vector of arthropod-borne viruses at the edges of villages (Boorman, 1960b), although it has so far not been incriminated as a vector in a natural epidemic. The biting cycle found at Jos resembled that found in southern Nigeria (Boorman, 1960b), with an abrupt peak of biting activity during the period from 1800 to 2000 hr. The closely related *A. africanus* was also taken in fairly large numbers; this species showed a biting cycle similar to that found elsewhere in Nigeria (Mattingly, 1949; Boorman, 1960b). This species is a proven vector of yellow-fever virus in East Africa (e.g., Smithburn, Haddow & Lumsden, 1949) and Zika virus has been isolated from wild-caught specimens (Dick, Kitchen & Haddow, 1952; Weinbren & Williams, 1958).

A. vittatus was taken biting man in fair numbers; the biting behaviour of this species in other parts of Africa has been discussed by Mattingly (1952). Over most of its range it appears to bite man freely. During the present investigation, sporadic biting activity occurred at most hours of the day and night, although none were taken from 0700 to 1000 hr. A sharp peak of activity was evident at sunset, during the period from 1800 to 2000 hr.; in this respect *A. vittatus* resembles most of the other *Stegomyia* species that have been studied. In one of the baited catches, a few examples of *A. vittatus* were taken, together with some of *A. luteocephalus*, in the centre of a small village, indicating that they will at least occasionally enter villages to bite. Early stages of *A. vittatus* and biting adults have also been found in the centre of a small village, Idanre, in southern Nigeria (author's observation, unpublished). No adults of this species were taken

during the spray-catches, however, although these were carried out in close proximity to the breeding site. Thus it seems probable that they do not enter huts to rest. Unfed females of *A. vittatus* were taken among grass and other vegetation in the hand-catches, but these adults may have been freshly emerged; no blood-fed adults were taken.

An interesting occurrence was the capture of *A. ingrami* at human bait, in fair numbers. This is a distinctive species and unlikely to be overlooked where it occurs; it appears to be a rare species in southern Nigeria. The numbers taken were too small to allow of an accurate biting cycle to be drawn, but the indications were that there was a peak of biting activity in the early morning and another, sharper peak at about 1800 to 1900 hr. Its importance in the epidemiology of the arthropod-borne viruses is unknown, but it is of interest that Uganda S virus was first isolated from a pool of mosquitos containing this species (Dick & Haddow, 1952).

The number of *A. aegypti* taken was small; but the results indicate that the biting cycle resembled that found in southern Nigeria (Boorman, 1960a).

The most numerous species, both in baited and hand-catches, was *C. uni-vittatus*. Attention has already been drawn to this species by Lumsden & van Someren (1953) in connection with a possible dry-season cycle of yellow-fever virus, although the species does not seem to have been tested for its ability to transmit virus. They found the species to be more common above than at ground-level; no catching was done above the ground in the present study. In both cases there was a peak during the period from 1900 to 2000 hr., but in the Jos catches there was also a peak in the early morning, just before dawn (0500–0600 hr.).

In assessing the importance of any of these species in the spread of arthropod-borne viruses, it must be remembered that the catches were conducted at the beginning of the rainy season; the relative abundance of any of these might be considerably changed at the height of the rains. The indications are, however, that the *Stegomyia* species most likely to be involved are *A. luteocephalus*, *A. africanus* and *A. vittatus*. All three of these could be involved in a man to man, or primate to man, cycle, since all are found both near to and far from human habitations. The commonest primates in the area appeared to be baboons (*Papio* sp.), pottos (*Erythrocebus patas patas*) and tantalus monkeys (*Cerco-pithecus aethiops tantalus*). *A. aegypti* and *A. ingrami* appeared to be of lesser importance, although the former could well be an important vector where there was a large mosquito population in a village.

The small number of *A. simpsoni* caught biting indicates that the species does not commonly bite man in the Jos area; the species was breeding freely around the sites of the catches. It is presumably of no importance in virus epidemiology.

Breeding behaviour of mosquito species.

Most of the larvae taken were found in habitats typical of their species. The single record of one larva of *Anopheles gambiae* from a bamboo pot is unusual. *Aedes aegypti* was found breeding in a rock hole on one occasion only; most of the plateau villages show a lack of domestic containers suitable for this species, and the usual larval habitat is probably tree holes, from which many records were obtained.

The rock-hole surveys yielded several points of interest regarding the breeding behaviour of *A. vittatus*. In general, shallow holes, particularly those which contained less than half an inch of water when full, seldom contained larvae; even when they did, the larvae did not usually complete their life-cycle before the water had dried up. Deep holes usually contained larvae of *A. vittatus*, particularly those where the water was clear and there was a layer of mud and a few dead leaves. In such cases all instars were commonly present together, as in hole no. 69. Clear water was not an essential, however, since holes nos. 3 and 4

continuously supported a population of all instars. Where an excess of decaying vegetation was present, a bacterial scum formed on the surface of the water; this did not prevent larvae from attaining the 4th instar where the scum was thin (hole no. 16), although no pupae were found in this hole. Larvae were not found where the surface was covered with a thick scum. In these cases, the holes supported a large population of predators, and it is not clear whether the bacteria or predators were responsible for the absence of larvae; no tests were made to elucidate this point.

The most important of the predators found were considered to be dragonfly nymphs and tadpoles; although these undoubtedly disposed of large numbers of mosquito larvae they did not prevent a flourishing population of all instars in most cases. This is seen in holes nos. 32, 33, 40 and 41. Predators were not found in those holes in which the water was likely to dry up: for example, holes 8, 9, 15 or 24. Both dragonfly nymphs and tadpoles have a relatively prolonged developmental period but their ability to survive desiccation does not seem to have been investigated.

Some of the holes studied, particularly hole no. 69, continuously supported a population of all stages of *A. vittatus*. This hole was dry on 25th May, had first- and second-instar larvae on 26th May, all instars of larvae on 27th May and second-, third- and fourth-instar larvae on 28th May. On the next day pupae were also present and after this all instars were present each day. This suggests the length of the larval stage in nature to be about four days, with five to six days for the complete life-cycle from egg to adult. From the observations made on this hole from the 26th to 29th May, and on holes nos. 2, 3 and 4 over the same period, it was deduced that the first and second instars occupied about 18–24 hours each; the third instar was rather longer, probably about 24–36 hours, and the fourth instar was of about the same duration. This is a slightly faster rate of development than that usually quoted for *A. aegypti*: Christophers (1960) quotes about 24 hours for each of the first three instars and slightly longer for the fourth instar. The length of life-cycle found in *A. vittatus*, five to six days, is somewhat longer than might be expected in a species where the breeding places are liable to frequent drying. The effect of this frequent drying is well seen in hole no. 6, where although conditions for breeding appeared favourable, pupae were found on only one occasion. Although larvae and pupae are unable to survive periods of complete desiccation, they can evidently survive for at least 24 hours in wet mud. This may be seen in hole no. 6, in which second-, third- and fourth-instar larvae were present on the 10th June. The free water had dried out the next day, but the hole had refilled by the 12th June and all instars were then present. Neither larvae nor pupae seemed able to survive a day of 'damp mud', however, since in no instance were larvae other than first or second instar recorded after a record of 'damp mud' on the previous day. It was considered, though not proven, that these first and second instars were not survivors but larvae that had hatched when the hole refilled with rain-water.

Laboratory studies.

The laboratory studies support many of the conclusions drawn from the rock-hole survey results. The length of duration of the pupal stage corresponds with that of other species of *Stegomyia*—Christophers (1960) gives references to work with *A. aegypti*, the usual figure being around 48 hours. It was apparent that although pupae could hatch successfully if kept damp, they could not survive drying for six hours, but probably much less than this period of desiccation is lethal. The resistance of larvae to drying was not tested fully but they are probably no more resistant than are pupae. Neither larvae nor pupae proved resistant to temperatures above 45°C., and in this respect resemble the early stages of *A. aegypti* (Farid, 1949). The larvae and pupae of *A. vittatus* thus seem to

show no special adaptations such as might be expected at first sight to enable them to survive at temperatures higher than normal in a habitat exposed to considerable solar irradiation and liable to dry out frequently. In these situations the temperatures of the mud remaining in the holes might be expected to rise beyond those shown to be lethal. Vanderplank (*in* Hinton, 1951) has found that the temperature of wet mud in a rock hole at Kaduna one afternoon in January was 40°C. The temperature of water in the rock holes was lower than expected, and agreed with figures quoted by Hinton; thus water in the rock holes is unlikely to rise above lethal temperatures. It is evident that this species is able to survive at least some of the dry season in the egg stage; this was deduced from the results of the survival of eggs in the laboratory. Kirk (1941) states that the larvae of *A. vittatus* could be recovered from débris in rock holes in the Sudan by soaking such débris in water; the larvae were presumed to have come from drought-resistant eggs. Hinton (1951) has suggested that the surface temperatures on exposed rock at Kaduna may rise to as much as 70–80°C. during the hottest parts of the day. Drought-resistant eggs may thus be exposed to very high temperatures. The survival of such eggs of *A. vittatus* subjected to high temperatures was not tested, but it is known that the eggs of *A. aegypti*, which also may survive the dry season in the egg stage, are killed above 50°C. It is possible that dehydrated larvae could survive the dry season in débris in the same manner as Hinton has shown the larvae of the Chironomid, *Polypedium vanderplanki*, to do, but first- and second-instar larvae only were recorded at an interval of 24 hours after a record of a 'dry' hole. This is well seen in the case of hole 68, on 6th to 9th June, inclusive.

The distribution of Aedes vittatus in Nigeria.

Mattingly (1952), discussing the distribution of this species in the Ethiopian region, remarks that it combines drought resistance with a marked ability to withstand low temperatures. Most of the Nigerian records are from localities having less than 60 in. rainfall; for all practical purposes, it may be said that it is distributed throughout the savannah areas wherever rocky outcrops suitable for its breeding occur. The single record from Lagos (Ikoyi) is of some interest; Lagos is just on the fringe of a belt of savannah-type country to the west, and various species of insects typical of savannah areas do occur in Lagos from time to time. Good examples of this come from the Lepidoptera; *Graphium pylades* (F.) (PAPILIONIDAE), a characteristic savannah species, is quite common, and *Acraea servona* Godt. (ACRAEIDAE), a dry-country species, has been recorded once from Ikoyi.

Formation of rock holes.

During April 1961, the opportunity was presented to examine rock holes in the vicinity of Eruwa, a small village in the Guinea Savannah belt (7° 33' N., 3° 25' E.). In this area, granite outcrops occur much as in the Jos area, and there are numerous rock holes. They are of slightly different shape from those on the plateau, being mostly rectangular, about 15 in. broad and 20 in. long by six in. to a foot deep. According to the villagers, they are made by grinding food. A cylindrical stone, some 15 in. long and about 3 in. diameter is rubbed back and forth on a smooth patch of granite; the same patch of rock is used on each occasion and this patch is used by one family only. There may be several grinding patches side by side, one for cassava, one for peppers, etc. The estimate given for grinding a hole some nine in. deep was 20 years. All stages in rock-hole formation were seen, from a barely perceptibly smoothed patch to the fully formed rock hole. The use of these holes is presumably discontinued when they become too deep for grinding. At the time of visiting, most of the unused holes were filled with water and had numerous dead leaves; almost all supported a population

of larvae of *A. vittatus*. Many of these granite outcrops have been used as defensive positions in inter-tribal wars, and there are numerous remains of broken pots and the evidences of human habitations. Some of these holes seem to be of great antiquity; one large slab of granite, much weathered, bore half a hole and had presumably split off from the main body of rock across a hole. These observations bear out Hinton's supposition that the rock holes in Northern Nigeria are formed by human, rather than by natural agencies. The site of the Vom rock-hole survey also exhibited signs of human habitation: circles of stones, possibly representing the foundations of huts, were seen.

Summary.

An account is given of a survey of the mosquitos of the Jos area of Northern Nigeria, during May-June 1960, particular attention being given to those species likely to be concerned with the transmission of arthropod-borne viruses. The area concerned is in the savannah belt at an altitude of about 4,000 ft., with numerous rocky outcrops. The dry season is fairly severe, and extends from about October to May; during this time, drinking water for the villages is obtained from deep wells. At the time of the survey, that is, at the beginning of the rainy season, the most abundant species were *Culex univittatus* Theo., *Aedes ingrami* Edw., *A. aegypti* (L.), *A. africanus* (Theo.), *A. luteocephalus* (Newst.) and *A. vittatus* (Big.). The biting cycles of these species are illustrated.

A survey was carried out of the breeding habits of *A. vittatus* in rock holes; those most favoured were about a foot across and six to nine inches deep, and appeared to be of human origin. Larvae appeared rapidly in the holes after the first rains, and predators, particularly dragonfly nymphs and tadpoles, were common in the larger rock holes. Under natural conditions, the duration of the life-cycle was about five days. The survival of the early stages under adverse conditions was investigated in simple laboratory experiments. At normal temperatures, the pupae did not survive desiccation for six hours, and, though the resistance of larvae to drying was not tested fully, it is considered that they are probably no more resistant than are pupae. Eggs survived desiccation for 10 but not for 18 weeks. Water temperature in exposed rock holes was not a factor limiting development, but the temperature of the exposed surface of dry rock holes was such that it appeared unlikely that eggs in these exposed situations would be able to survive the dry season. It was suggested that there may be collections of water in sheltered tree holes or rock holes which support a small but more or less continuously breeding population; this, combined with the ability of the eggs of *A. vittatus* to withstand desiccation for at least ten weeks at normal temperatures would enable the species to survive the relatively prolonged dry season.

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ADMIXTURE OF MALATHION AND LINDANE WITH BAGGED MAIZE.

By W. M. GRAHAM and S. P. L. KOCKUM.

A decade ago, the major insect pest of stored maize in Kenya was the weevil, *Sitophilus oryzae* (L.). Experimental work was primarily directed to reducing the loss caused by this insect, and the consequent recommendations have resulted in large savings.

The use of lindane (γ BHC) in farm maize-cribs as recommended by Kockum (1953, 1958) has been readily accepted by European farmers, and the maize delivered from this source is virtually weevil free. Approximately 1,000,000 bags entering central stores are produced by African growers and receive no insecticidal treatment. Immediate routine fumigation of all incoming maize is vital. *S. oryzae* has seldom been a post-fumigation pest in Kenya, but as the weevil problem in central stores has been largely solved, several secondary pests have assumed greater importance; for example, the rust-red flour beetle, *Tribolium castaneum* (Hbst.), the saw-toothed grain beetle, *Oryzaephilus surinamensis* (L.), and the moth, *Cadra cautella* (Wlk.), have become the major pests of bagged maize. A post-fumigation infestation by *T. castaneum* is usually established within 24 hours of the removal of fumigation sheets, and a large population builds up in three to four months. Store spraying and layer dusting appear to have had little effect in controlling these three insect pests.

Ideally, all maize entering central stores should have an insecticidal dust admixed. If the dust were admixed by the traders who purchase the maize grown by Africans it would generally stop the weevil infestation about two months before it is now halted by fumigation. Furthermore, maize with an admixed insecticide should be adequately protected from post-fumigation infestation by secondary pests. Previous work in Kenya by Le Pelley & Kockum (1954) on admixture of insecticides was directed only to the control of *S. oryzae*, and the resultant recommendation has remained, since 1954, at 1 part per million (p.p.m.) of lindane (the recommended diluent being 8 oz. of diatomite per bag). Admixture has never been done on a large scale in Kenya, but since such a practice was contemplated, experimental information was required to determine which treatment would under local conditions give protection from weevil infestation and from reinfestation by secondary pests. The quantity of eight oz. of diluent in a bag of 200 lb. of maize has been found to be objectionable, so in this experiment, designed to test lindane and premium-grade malathion, only four oz. of diluent was used.

Experimental plan.

The experiment was located in a large maize store used by the Nyanza Province Marketing Board. The store is a converted hangar situated several hundred yards from Lake Victoria at Kisumu, and the climate provides near optimum conditions for activity and abundance of common pests and therefore a rigorous test of any insecticide. Half of the floor space of the store was available for the experiment, and the remaining space was used for ordinary storage of maize (two stacks containing just over 10,000 bags).

Nine treatments were used: — untreated control (C); kaolin alone (K); diatomite alone (D); 0.125% lindane in diatomite (DL₁₂₅); 0.250% lindane in diatomite (DL₂₅₀); 0.500% lindane in diatomite (DL₅₀₀); 0.16% malathion in kaolin (KM₁₆); 0.32% malathion in kaolin (KM₃₂); 0.64% malathion in kaolin (KM₆₄). Treatment C had no dust admixed, and all the other treatments had four ounces of dust admixed per 200 lb. of maize. DL₁₂₅, DL₂₅₀ and DL₅₀₀ are equivalent to about

1.5, 3.0 and 6.0 p.p.m. of lindane, respectively. KM_{16} , KM_{32} and KM_{64} are equivalent to 2, 4 and 8 p.p.m. of premium-grade malathion, respectively.

Maize was used which had been in storage for several months, yet which was free of insecticides. One hundred and eighty bags of maize were emptied on a concrete floor and thoroughly mixed by shovelling until the maize was assumed to be of uniform quality and moisture content. The grain was rebagged and placed at random in nine groups of 19 bags; the remaining maize was excluded from use. A group of 19 bags of uniform maize was then emptied on to the floor, and a weighed quantity of dust (76 oz.) was admixed by shovelling. When refilling the bags, 18 were used and the last bag to be filled was excluded. Each treatment was carried out in the same way.

The experiment was arranged in a two-layered, 9×9 Latin square, so that the upper and lower bags of any one pair had the same treatment. One hundred and sixty-two bags were weighed and placed on dunnage so that each pair of bags was about 18 in. from any other pair. The intention was to remove the upper bags for sampling after four months, and to keep the lower layer for an extended time, depending on the data obtained at the first sampling.

In addition to the Latin square, there were two small stacks referred to as 'trader's stacks' (fig. 1). Each stack was made up of 50 bags of maize which had been bought and treated by a maize trader.

The Latin square and the trader's stacks were placed in position by mid-January (the store was otherwise empty and fairly clean). One trader's stack and

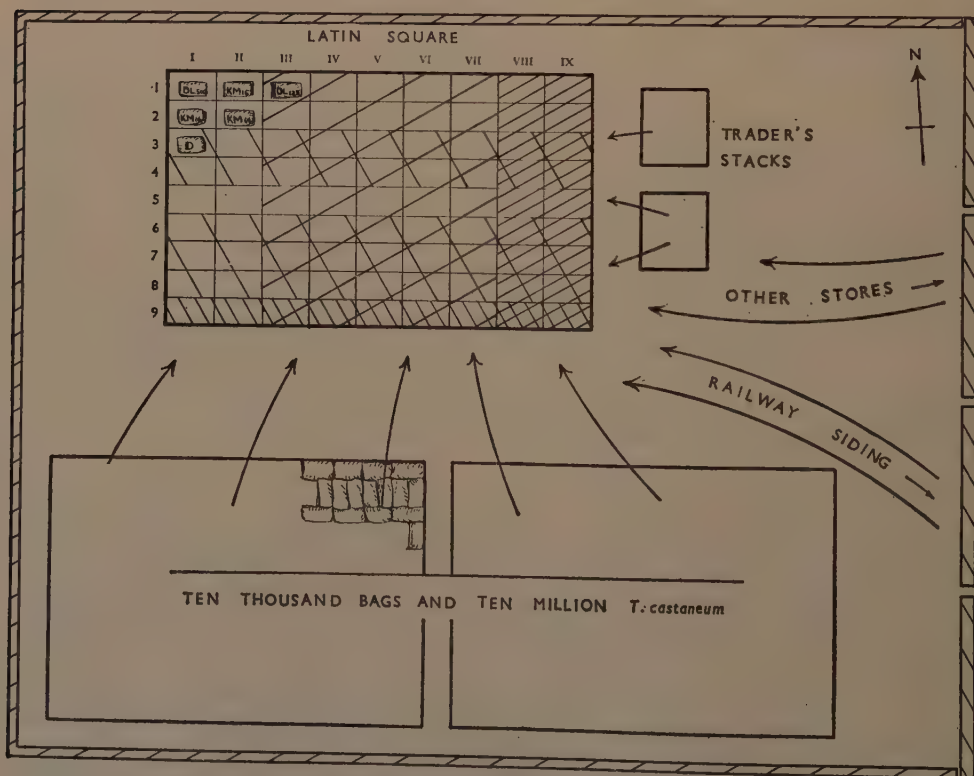


Fig. 1.—Floor plan of the hangar showing position of the Latin square (rows I-IX and columns 1-9), the trader's stacks, and the two large stacks. Suspected sources and directions of cross-infestation are indicated by a number of arrows.

the Latin square were fumigated in position with methyl bromide gas on 15th January. By 15th March, most of 10,000 bags of infested maize had been built into two large stacks 20 ft. from one side of the Latin square (fig. 1). A very heavy population of secondary pests (not *Sitophilus*) built up in the 10,000 bags, and these large stacks were of necessity fumigated on 1st May. The upper layer of the Latin square was sampled on 15th May (4-month sampling). The lower layer of the square, both trader's stacks, and all other maize in the store was fumigated on 22nd June. The second sampling (5½-month sampling) was done shortly after 22nd June.

Weight-loss figures were obtained by carefully weighing bags when they were placed in position and again at the end of the experimental period. A beam scale was used, as experience had shown that it gave less variable results than the generally employed platform scale. Two separate weighings were averaged to obtain both initial and final bag weights.

Moisture content was measured at the beginning and end of the experiment.

At the 4-month sampling, the upper bags of the Latin square were lifted, turned over, and placed beside the bottom bags. A count was then made of moth cocoons found on the line of contact of the two bags. All the cocoons were assumed to be those of *C. cautella*. The presence of a cocoon, whether it contained a prepupal larva, a pupa or a pupal case, was considered to indicate that a moth larva had been living in one of the two bags of maize. It was assumed to be likely that any larvae which might have migrated would become distributed uniformly between sacks, irrespective of their content.

After the 81 upper bags had been weighed, and samples for moisture-content determinations taken, each bag was sieved and the dust collected. The sack sieve used was built on the pattern of the cocoa sieve used in West Africa, and has a screen of five meshes per in. It was not available at the start of the experiment, and consequently the 162 bags of maize used were not sieved before being treated, nor was there a measure of the dust in the bags after mixing by shovelling.

An insect-trapping technique was employed which recovered from each frass sample some 95 per cent. of the living adults of *T. castaneum*, as well as a representative proportion of the living adults of *O. surinamensis* and *S. oryzae*. The thousands of trapped insects were killed, and were later counted at the laboratory in Nairobi. There were very few living adults of *S. oryzae*, so the 81 frass samples were fumigated and sent to Nairobi where the dead weevils (a few had been living at the time of sampling) were screened and winnowed from the frass, and were counted.

The sampling was simplified for the 5½-month sampling. Each of the lower bags was weighed twice and sieved. The dust was weighed, but insects were not trapped (very few living insects were seen as the bags had been fumigated three weeks before the final sampling).

Following the 5½-month sampling, a subsidiary experiment was completed, which related to the uniformity obtained by mixing grain by shovelling, the recovery of admixed diatomite and kaolin from clean and dusty maize by sieving, and other related points. The results of this work are employed to facilitate the interpretation of the results of the main experiment.

Results.

Tables of individual readings are not given, but mean values and the results of the statistical analyses are given for most sets of data. The results of the analyses of variance are summarised in Table I (see p. 732).

Losses in weight.

The weight-losses of bags have been calculated as the difference between initial and final weights of bags, minus an amount relative to natural drying; the

resultant net weight-losses are expressed as percentages of the initial net weights of bags. Moisture-content data, consisting of several thousand determinations, is not presented in detail. The drying during 4 months and 5½ months was the same for all treatments, rows and columns (analysis of variance). After four months, all upper bags had lost moisture equivalent to about 0.50 per cent. of the initial net bag weight; after 5½ months, the lower bags had lost moisture weighing 0.54 per cent. of the initial net bag weight.

The analysis of variance for the weight-loss data from the 4-month sampling (upper bags) shows a very high significance ($P < 0.001$) for treatments and columns, and also a high significance ($P < 0.01$) for rows (Table I). In the analysis, the angular transformation generally employed when using percentage data was not used, as it is doubtful whether such a transformation is of value when all the percentage values are so small. The mean percentage weight-losses and their *t*-test interrelations for treatments, rows (I-IX), and columns (1-9) at the time of the 4-month sampling are:

Treatments

KM ₆₄ 0.173	KM ₃₂ 0.221	KM ₁₆ 0.264	DL ₅₀₀ 0.330	DL ₂₅₀ 0.350	DL ₁₂₅ 0.439	D 0.461	K 0.485	C 0.497
---------------------------	---------------------------	---------------------------	----------------------------	----------------------------	----------------------------	------------	------------	------------

Rows

VIII 0.284	IX 0.311	VII 0.318	VI 0.364	I 0.368	V 0.379	III 0.387	IV 0.397	II 0.413
---------------	-------------	--------------	-------------	------------	------------	--------------	-------------	-------------

Columns

1 0.256	2 0.293	4 0.321	3 0.334	7 0.369	5 0.376	8 0.406	6 0.406	9 0.458
------------	------------	------------	------------	------------	------------	------------	------------	------------

The above rectangles enclose those treatments not shown to be significantly different from one another (*t*-test), and those values not enclosed by the same rectangle are significantly different. The difference between two means required for significance at the 5 per cent. level is 0.072. Reference to fig. 1 will clarify the relation of rows and columns to the position of the Latin square in the store. The reason that rows VII, VIII and IX have smaller weight-losses than rows I to IV may be because the weighing of bags began with row IX and finished 8-10 days later with row I. The highly significant differences between columns are much more easily related to the orientation of the square; columns 8 and 9 are close to the heavily infested large stacks of maize, and the heavier losses on this side of the square are indicative of a heavier cross-infestation. During the middle two months of the first four months of the experiment the large stacks, containing some 10,000 bags, were supporting a population of *T. castaneum* estimated at more than 10,000,000 living adults. There was no difference in the weighing-out times of the nine columns.

The data from the 5½-month sampling were very similar to those of the 4-month sampling.

Weights of dust.

The dust collected from the sack sieve (no. 5 screen) consisted of chaff, grain fragments and dust, insect faeces, and insecticidal dust. An attempt was made to remove the coarser portions by using no. 10 and no. 20 screens, but the extra work did not give more refined data. The dust weights were corrected by deducting the weight of insecticidal dust which was suspected (on the basis of a subsidiary experiment) to have passed through the sack sieve into the gross dust sample. The correction did not alter the relative positions of the means of the eight treatments other than the control. The net weights of dust were then expressed as percentages of the net bag weights.

In the analyses of variance for both sets of data (upper and lower bags of the Latin square), the treatment differences were very highly significant (Table I). The results of the treatment *t*-tests are shown as before:

Upper bags (4-month sampling)

KM ₄₄ 0.051	KM ₃₂ 0.069	KM ₁₆ 0.077	DL ₅₀₀ 0.091	DL ₂₅₀ 0.118	DL ₁₂₅ 0.141	D 0.169	K 0.220	C 0.251
---------------------------	---------------------------	---------------------------	----------------------------	----------------------------	----------------------------	------------	------------	------------

Lower bags (5½-month sampling)

KM ₃₂ 0.057	KM ₆₄ 0.058	KM ₁₆ 0.110	DL ₅₀₀ 0.133	DL ₂₅₀ 0.138	DL ₁₂₅ 0.173	D 0.179	K 0.237	C 0.289
---------------------------	---------------------------	---------------------------	----------------------------	----------------------------	----------------------------	------------	------------	------------

The differences required between means for significance at the 5 per cent. level are 0.022 for the upper bags, and 0.028 for the lower bags.

Analysis of the 4-month sampling data did not show rows and columns to have even an indication of significance, but rows and columns of the lower bags had an indication of significance approaching the 10 per cent. level (Table I). Row IX and column 9 of the lower bags had the highest percentage dust means.

Number of living adults of T. castaneum.

Only the 4-month sampling data include counts of insects. The analysis of variance for *T. castaneum* (and other insects) is based on a square-root transformation. Treatments, rows and columns were all found to be significant at the 1 per cent. level (Table I).

In the following presentation (by the use of rectangles) of the *t*-test for treatments the means of the numbers of *T. castaneum* are given, and below these actual numbers are the means of the square-root values:

Treatments

KM ₆₄ 380.8 17.70	KM ₃₂ 429.9 20.44	DL ₁₂₅ 605.6 24.19	DL ₂₅₀ 621.7 24.46	D 737.4 26.49	KM ₁₆ 789.3 26.76	DL ₅₀₀ 778.6 27.07	K 923.9 30.13	C 1198.8 34.12
------------------------------------	------------------------------------	-------------------------------------	-------------------------------------	---------------------	------------------------------------	-------------------------------------	---------------------	----------------------

Rows (means of square-root values only)

II 21.62	I 22.09	IV 22.98	III 24.98	V 25.99	VI 26.62	VII 26.81	IX 28.67	VIII 31.61
-------------	------------	-------------	--------------	------------	-------------	--------------	-------------	---------------

Columns (means of square-root values only)

2 20.83	1 21.18	5 23.70	3 24.71	6 25.03	4 25.20	7 27.43	8 27.73	9 35.55
------------	------------	------------	------------	------------	------------	------------	------------	------------

The difference between two means required for significance at the 5 per cent. level is 3.62.

Number of living adults of O. surinamensis.

The numbers of *O. surinamensis*, unlike the numbers of *Tribolium*, do not represent a complete count, as we had no efficient technique for trapping, but the ratios of the numbers found in the traps are assumed to be correct and the results of the analysis of variance were very similar to those for *T. castaneum*. The analysis of variance showed treatments and rows to be highly significant, and columns to be significant (Table I).

TABLE I.

Results of the analyses of variance for 8 sets of data.

Sampling data	Variance ratio (<i>F</i>)			Comments
	Treat-ments	Rows (I-IX)	Columns (1-9)	
Percentage dust weight 4-month sampling	78.21***	1.34	1.21	
Percentage dust weight 5½-month sampling	62.87***	1.82	1.97	Rows and columns not significant, but approaching 10%
Percentage weight-loss 4-month sampling	22.46***	2.98**	6.18***	
Percentage weight-loss 5½-month sampling	19.69***	2.93**	3.77**	
<i>T. castaneum</i>	14.55***	6.41***	11.82***	
<i>O. surinamensis</i>	7.32***	4.91***	3.40**	
Moth cocoons	13.74***	(1.14)	(1.51)	A bracketed ratio signifies that the residual variance has been divided by the factor variance
<i>S. oryzae</i> (living plus dead)	4.25***	4.92***	(1.43)	

The values of *F* required for given levels of significance are as follows: 10 per cent. (*P*=0.1), 1.99; 5 per cent. (*P*=0.05), 2.12; 1 per cent. (*P*=0.01), 2.85; 0.1 per cent. (*P*=0.001), 3.90. The ratios in the table that reach a high level of significance (*P*=0.01) are indicated by two asterisks, and those that reach a very high level (*P*=0.001) by three asterisks.

Number of living and dead adults of S. oryzae.

As with the *O. surinamensis*, the trapping technique used was not an effective means of collecting *S. oryzae*. Furthermore, in comparison with the numbers of other beetles, very few weevils were seen. The scarcity of *S. oryzae* was expected, as all the experimental maize except that in one trader's stack had been fumigated at the outset with methyl bromide, and reinfestation by weevil under such conditions is generally slight. The stacks containing 10,000 bags had been fumigated

in another store two to three months earlier and were not noticeably infested with *S. oryzae*. The number found in the control (9-bag total) was 110 and no other treatment contained more than a total of 4.

At a later date, all the adults of *S. oryzae* were extracted from the frass samples for counting, and the numbers so obtained were added to those initially counted as living to give a living-plus-dead weevil count. The analysis of these data showed both treatments and rows, but not columns, to be very highly significant (Table I). The results of the *t*-test are:

Treatments

DL ₂₅₀	DL ₅₀₀	DL ₁₂₅	D	KM ₁₆	K	KM ₆₄	KM ₃₂	C
361	401	485	518	520	516	563	596	832
18.65	19.71	21.63	22.23	22.26	22.45	23.36	24.15	28.14

As before, the first values shown under treatments are the means of the actual numbers, and the second values are the means of the square roots (used for analysis). The difference required for significance at the 5 per cent. level is 3.74.

Counts of moth cocoons.

The analysis of variance using square roots of the actual cocoon numbers showed treatments to be very highly significant (Table I). That the differences within rows and columns were not significant supports the assumption that larval migration was not the determining factor in the distribution of the cocoons found between the upper and lower bags. It can be safely assumed that most of the larvae developed in the bags where their cocoons were counted.

The presentation of the results of the *t*-test is the same as in previous cases:

Treatments

KM ₆₄	KM ₃₂	D	DL ₁₂₅	DL ₅₀₀	KM ₁₆	DL ₂₅₀	K	C
25.3	46.1	90.3	101.4	110.4	127.7	148.1	193.7	194.3
4.77	6.48	9.20	9.82	10.22	11.01	11.96	13.65	13.82

The difference required between means for significance at the 5 per cent. level is 2.32. The transformed means of rows and columns were not shown to be significant in the analysis of variance.

Discussion.

The uncorrected weight-loss data from the 4-month sampling showed the KM₆₄ treatment (8 p.p.m. malathion) to be the most effective, yet apparently to have permitted a weight-loss of 0.673 per cent. (1.346 lb. per bag). Weight-loss is determined by a complex of factors; drying, grain metabolism, mould metabolism, and insect feeding. The loss due to drying was calculated to be 0.50 per cent. of the initial net bag weight. After the relevant correction was made for the KM₆₄ mean, the weight-loss was still 0.173 per cent. whereas it might have been expected, judging from insect numbers and dust weights, to be nearly *nil*. Since the maize had a moisture content of just over 12.50 per cent. when the experiment began, and since the mean store temperature was above 25°C., it can be assumed that mould respiration contributed significantly to the weight-loss (Anderson & Alcock, 1954). The weight-loss of 0.173 per cent. in four months could be wholly attributed to mould metabolism. This hypothetical explanation of the KM₆₄ weight-loss excludes the possibility of a loss relative to insect feeding.

The justification for this approach was found in a comparison of the 5½-month data with the 4-month data, shown in fig. 2, where the percentage weight-loss is plotted against the percentage dust recovered by sieving. After making a separate deduction for drying (0.54 per cent. of the initial bag weight as against the 4-month correction of 0.50 per cent.) the weight-loss in the lower bags was about 0.19 per cent. greater than the loss in the upper bags. The moisture difference of 0.04 per cent. between the 4-month and 5½-month samples is as near factual as any electric moisture meter determination can be. The discrepancy of 0.19 per cent. can only be explained by assuming the existence of factors which increase weight-loss, but do not increase the amount of dust; namely, grain and mould metabolism. The 0.19 per cent. increase in weight-loss attributed to metabolism of grain and mould is not necessarily due to respiration during only the last 1½ months of the experiment; the grain in the lower bags was of a slightly higher moisture content and therefore had a higher rate of metabolism during the first four months. Methyl bromide gas is known to act as a fungicide, so it is possible that the rate of respiration was much greater in the last months of the experiment than during the earlier post-fumigation period.

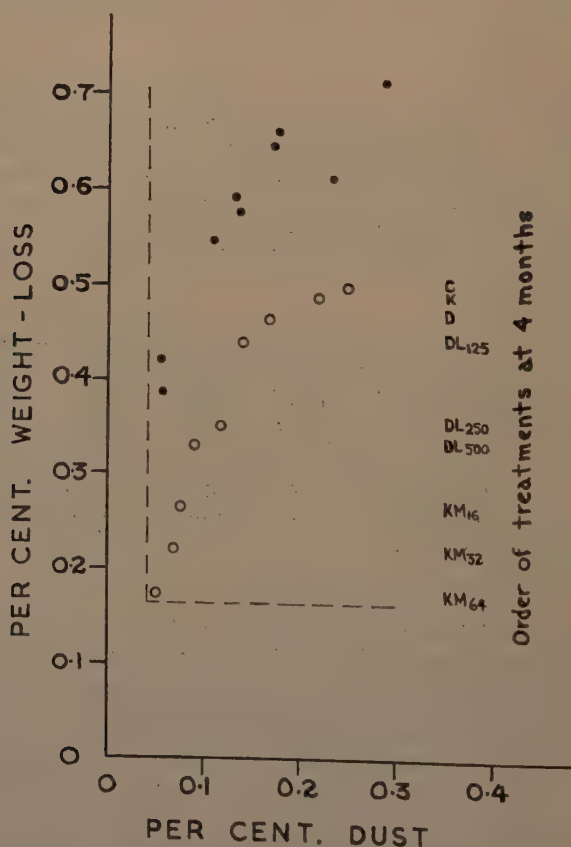


Fig. 2.—The mean percentage weight-loss per bag and mean percentage dust per bag for the nine treatments used in the Latin square. Circles refer to upper bags (4-month sampling) and dots refer to lower bags (5½-month sampling). Broken lines show the estimated zero per cent. axes relevant to the 4-month sampling data.

Another indication of mould inhibition (suppressed weight-loss) is to be seen in the downward trend of the line in fig. 2, which is possibly related to the fungicidal effect of *Tribolium* secretions reported by van Wyk, Hodson & Christensen (1959).

Dust-weight measurement had a lower experimental error than the weight-loss data derived from weighing full bags on a beam scale. Percentage dust is a more direct measure of treatment efficacy, as dust cannot increase without increased insect feeding. Judging by the amount of dust present, malathion at a rate of 8 p.p.m. was the best treatment, yet apparently allowed 0.051 per cent. dust to accumulate. The dust recovered by sieving falls into several categories:— (a) chaff and broken grains due to the actual mechanics of the sieving; (b) shrivelled and underdeveloped maize grains that are less than one-fifth of an inch in diameter; (c) frass present prior to the initiation of the experiment; (d) added insecticidal dusts; and (e) frass produced by insects during the experiment period. The sack sieve was not available at the beginning of the experiment, therefore we have no measure of categories (a), (b) and (c). As described in the "Results", all the dust data were corrected for the presence of insecticidal dust (d). There was no increase in the average percentage dust for malathion at 8 and 4 p.p.m. between four months and 5½ months; the other seven treatments showed increases in percentage of dust ranging from 0.010 to 0.042 (fig. 2). Since KM_{64} and KM_{32} apparently gave complete protection during the last five weeks of the experiment, it can be accepted that the protection for the first four months was also complete; the dust in the KM_{64} bags (mean of 0.051%) was not frass produced by insects during the experimental period.

In fig. 2, the zero per cent. dust line, and zero per cent. weight-loss line related to the 4-month sampling have been roughly estimated and shown as broken lines; there is little experimental data to support the actual positions given to these lines, and they are shown only to illustrate more clearly the most likely relative protection provided by the nine treatments.

It should be remembered, especially when considering the data regarding living insects, that the protection provided by both lindane and malathion treatments was undoubtedly greater during the first three months, the most important period, than after four months. Both insecticides are relatively volatile or unstable. Gunther, Lindgren & Blinn (1959) reported that the half-life of admixed malathion dust at 8 p.p.m. is about five months. Strong & Sbur (1960) showed that a grain moisture content of about 14 per cent. was critical in regard to malathion persistence, and that the maximum safe level was about 12 per cent. The majority of the insects present, and nearly all of the measured damage at the time of the 4-month sampling, can be assumed to have been related to extremely heavy cross-infestation during the third and fourth months of the experiment; as the insecticides became less effective, cross-infestation reached a maximum.

Although weight-loss and dust figures are often difficult to interpret, being measures of numerous interrelated factors, insect numbers are even more complex, on account of population fluctuations, and movements relative to temperature, moisture, or crowding. The analyses of numbers of *T. castaneum*, *O. surinamensis* and moths, generally all showed that malathion treatments at an initial 8 and 4 p.p.m. gave superior protection; 1.5, 3.0 and 6.0 p.p.m. of lindane and 2 p.p.m. of malathion were similar to one another; diatomite was hardly distinguishable in effectiveness from diatomite plus lindane; and kaolin and control were inferior to the other seven treatments.

The significance of cross-infestation from the heavily infested stacks containing 10,000 bags is very evident from the analysis of variance for *T. castaneum*, which showed highly significant differences between the columns. In column 1 (furthest from the source of cross-infestation) there was a mean number of 485 adults of *Tribolium* per bag, and in column 9 (nearest the large stacks) a mean of 1,327. The column differences for *Oryzaephilus* gave a similar picture of cross-infestation.

Row differences for *Tribolium* were also highly significant and possibly were determined by the direction of cross-infestation. The greatest numbers of *Tribolium* were found in rows VI to IX; near the trader's stacks and the doors of the stores (fig. 1).

Possibly the most striking presentation of the data is found in fig. 3 (a) where the (square-root) treatment means for *T. castaneum* are plotted against the treatment means of percentage dust. Since in this experiment most of the dust was

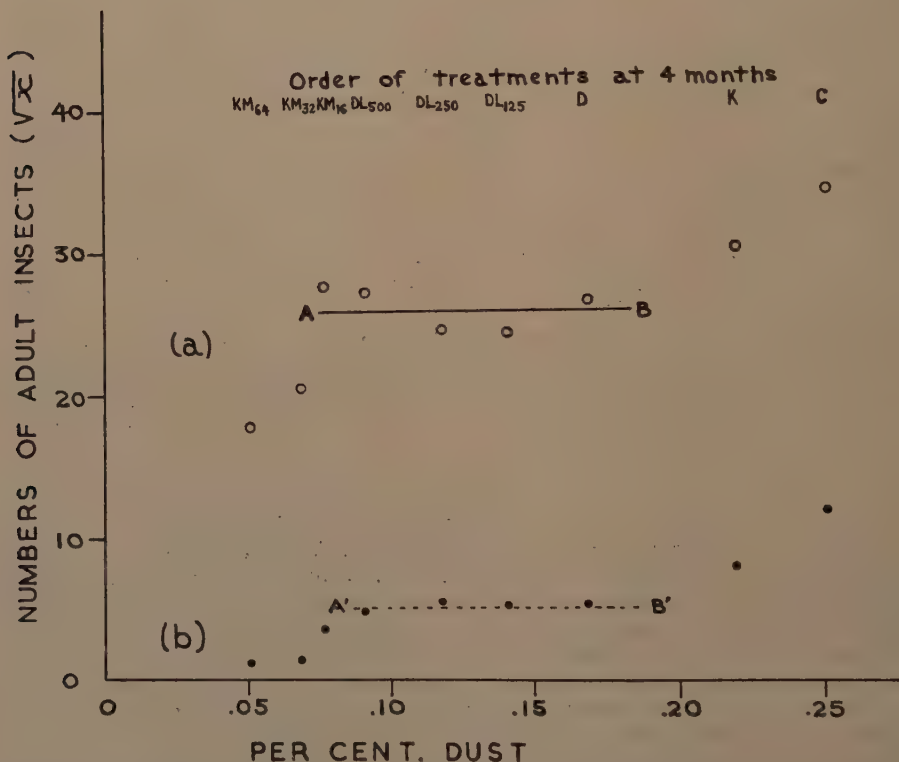


Fig. 3.—Means of the transformed numbers of *T. castaneum* (a), and *O. surinamensis* (b) related to the mean percentage dust per bag in the nine treatments of the Latin square. Lines AB and A'B' are described in the text.

probably produced by *T. castaneum*, an approximately linear relationship between numbers of *Tribolium* and percentage dust could be expected: there is obviously no such simple relationship. A horizontal line (AB) has been drawn by eye through five points of fig. 3 (a), and can be considered to be the level of adults of *T. castaneum* per bag that was determined solely by cross-infestation from the neighbouring 10,000 bags. Such a level of *T. castaneum* per bag should have been the same for all treatments if deaths and breeding were constant; such a hypothetical level of adult numbers is here referred to as the cross-infestation level and is denoted on the graph as line AB. The *t*-test for the data for *T. castaneum* showed no significant difference between the five points around line AB (KM₁₆;

DL₅₀₀; DL₂₅₀; DL₁₂₅ and D). On the basis of the data given by Parkin (1960) it may be inferred that lindane at less than 10 p.p.m. will give negligible control of adults of *Tribolium*, and that malathion under 2 p.p.m. (KM₁₆) will also be ineffective in killing adults. His work also showed that an initial population of beetles would have failed to increase at any concentration of lindane above 1 p.p.m., and that malathion above 0.5 p.p.m. and lindane at 2.5–5.0 p.p.m. prevented breeding of *Tribolium*. In the presentation of the data in fig. 3 (a) the cross-infestation level is maintained because the five treatments concerned caused no death of adults, and there was no increase in the numbers as breeding was prevented. Earlier work by Le Pelley & Kockum (1954) emphasised the insecticidal value of diatomite, which also is assumed to have prevented breeding without having caused death. The variation in percentage dust along the line AB can be explained by the variable degree of insect activity; the adult beetles in KM₁₆ are assumed to have survived but to have eaten very little; at the other end of the line AB, the same numbers of adults of *T. castaneum* in the D bags are assumed to have been eating normally and possibly to have laid eggs which hatched, but the larvae or pupae are assumed to have died. KM₆₄ and KM₃₂ had many fewer living adults (below the cross-infestation level) because many of the adults of *Tribolium* died soon after they entered the bags. At the other end of the line, the K and C treatments neither killed adults nor prevented breeding, therefore the population rose above the line AB. The data appear to give a very good field confirmation of the conclusions reached by Parkin. The interpretation can equally well be extended to include numbers of *O. surinamensis*, if it is assumed that they are large enough to influence the dust weights significantly (fig. 3 (b)).

The data of living adults of *S. oryzae* indicate most clearly that any dust treatment will to a large extent control weevil reinfestation. The analysis of the living-plus-dead figures illustrates that malathion was not more effective than lindane as was weakly suggested by the living weevil figures. This analysis also shows all the treatments other than the control to have been fairly effective. Although the weevil cross-infestation pressure was low in this experiment, it can be accepted that malathion will generally give sufficient protection. Lindgren, Krohne & Vincent (1954) reported an experiment with dust admixture in which 8 p.p.m. of malathion was effective against *S. oryzae* for 6–7 months, but stated that oviposition prior to death was not completely checked. Parkin (1960) reports that malathion admixed with wheat at a rate of 2 p.p.m. gave 100 per cent. kill of *S. oryzae* in 13 days, but 4–8 p.p.m. was required to prevent breeding. The statistical analysis of living-plus-dead figures shows the lindane treatment slightly but consistently better than the malathion; this may only be related to the fact that the lindane, due to its fumigant action, more effectively prevented the emergence of adults during the week prior to the initial fumigation. Columns were not significantly different in the analysis of variance; this is in agreement with the observation that there was no cross-infestation pressure from the two large stacks. Rows showed a highly significant difference which can be roughly related to the unfumigated trader's stack (fig. 1), which was heavily infested with *S. oryzae*.

The economics of admixture are not fully shown by this experiment as it gave information on the economics related only to the prevention of post-fumigation infestation in central stores. In fact, the information is only indicative, as the results of a bag experiment such as this one are not fully applicable to storage of bags in large stacks. The quantitative savings (dust weight and weight-loss summed) determined by measuring the difference between treatments C and KM₆₄ are greater than 0.5 per cent. at four months and of a monetary value of more than double the cost of the insecticide used. The weight-loss results also suggest that the losses due to mould metabolism may be of a magnitude to justify preventive measures.

Summary.

An experiment was done in Kenya to test several formulations of lindane and malathion dusts as protectants of bagged maize, with which they were mixed, against reinfestation (post-fumigation infestation) by store pests. The experiment was in the form of a 9×9 Latin square employing 162 bags. Sampling initially and after 4 and $5\frac{1}{2}$ months gave data on bag weight-losses, dust present per bag, numbers of adults of *Tribolium castaneum* (Hbst.), *Oryzaephilus surinamensis* (L.), *Cadra cautella* (Wlk.) and *Sitophilus oryzae* (L.), and moisture-content changes.

The dust weights (which were expected to be the most reliable measure of treatment efficacy) showed that there was very little difference between malathion admixed at 8, 4 and 2 parts per million. The other six treatments were far less satisfactory and all were significantly different from one another, with the following order of values: lindane at 6, 3 and 1.5 p.p.m., diatomite, kaolin and control. All the other data generally supported these results.

The extreme importance in this experiment of cross-infestation from nearby stacks has been shown in the analysis and has been illustrated by plotting numbers of *T. castaneum* against dust-weight data. Interpretation of this curve has lent support to work with lindane and malathion published by E. A. Parkin in 1960.

The results emphasise the significance of the contribution of grain and mould metabolism to the total bag weight-losses. Some estimates are made of the savings relevant to the admixture of malathion.

Acknowledgements.

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FEEDING BEHAVIOUR OF ADULTS OF *PIERIS BRASSICAE* (L.) IN A LABORATORY CULTURE.

By W. A. L. DAVID and B. O. C. GARDINER

*Agricultural Research Council Unit of Insect Physiology,
Cambridge.*

In the wild, *Pieris brassicae* (L.), the large white butterfly, feeds on a variety of flowers and such crop plants as field beans, clover and lucerne are said to be especially attractive (Frohawke, 1934). It would be inconvenient, however, to maintain a large laboratory culture on natural flowers and an alternative method of feeding the insects has been developed since, although unfed females mate, they produce relatively few eggs before they die.

It has been known since the experiments of Lubbock (1882) that insects will visit suitably coloured pieces of paper, and Ilse (1928, 1941) has shown that in the case of *P. brassicae* the reaction is spontaneous and does not depend on previous training. For these reasons it seemed likely that the adults could be fed successfully on artificial flowers containing a tube of honey solution. In fact, however, the results with four stocks, newly established from eggs or larvae collected in the field, made it quite clear that the majority of the adults ignored the artificial flowers and it was only after a few generations in captivity that a strain which fed readily was obtained.

The first few generations of adults reared in captivity seemed preoccupied with the urge to escape from the cage and in their incessant fluttering against the walls many of the insects broke off the tips of their wings—which does not happen with the adults of a well-established culture. Yet if the adults of these new stocks are given natural flowers they will feed, as they also will after about three days of starvation when held on the artificial flowers with their tarsi touching the honey solution. Sometimes, to induce feeding, it is necessary, in addition, to uncoil the insects' proboscides so that they dip into the solution.

By collecting the eggs laid by insects which have not fed or which have been induced to feed on the artificial flowers or which have fed voluntarily on natural flowers, the culture can be continued. And if this procedure is repeated in the next two or three generations a stock is obtained in which a high percentage, if not all, of the adults feed of their own accord on the artificial flowers.

It seems probable that it was mainly their failure to obtain a stock of adults adapted to the breeding conditions that led to the difficulties which other workers have reported in maintaining a culture of *P. brassicae* (Blunck, 1935, personal communication, 1956; Way, Smith & Hopkins, 1951). However, it is clear from the results given later in this paper that many factors can influence the feeding of caged butterflies and some may be of vital significance during the critical period of adaptation.

In reading this paper it should be remembered that the results relate to a stock in which the adults were adapted to life in cages.

Equipment and methods.

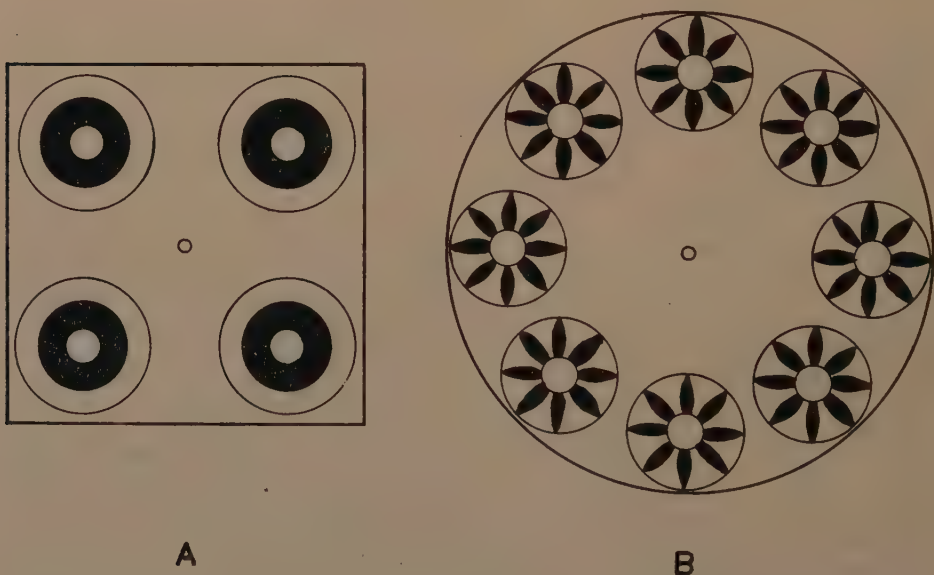
The insects were bred and the tests were carried out in the glasshouse previously described or in constant-temperature rooms, and the same cages and methods have also been employed (David, 1957; David & Gardiner, 1952, 1961).

Unless stated otherwise, the feeding tests were run in the large type of adult cage measuring $40 \times 30 \times 36$ in. high. As an alternative, the smaller type cage measuring $13 \times 13 \times 18$ in. high was used in some experiments. In addition to the daylight there was a 500-watt tungsten filament lamp in an enamelled reflector over each cage as there was over the stock cage.

It is easy to design a variety of artificial flowers differing in construction, colour, size and pattern but the suitability of any one type for feeding the insects can only be determined by experiment.

From a practical point of view, ease of construction, cleaning and filling must be considered. And unless the honey solution is entirely changed each day the volume in the flowers should be such that a large part is drunk during the day and replaced by fresh solution. In addition, the flowers must attract the insects which must then be able to find and drink the honey solution without difficulty.

Two main types of artificial flower plates were finally designed. The standard patterns used are illustrated in figs. 1A and 1B, and various modifications are described in the sections dealing with particular experiments.



Figs. 1A and 1B.—The two main types of plates of standard artificial flowers used in the tests. Detailed descriptions are given in the text and various modifications are also described.

The type-1A flower was made from a Perspex plate $\frac{1}{8}$ in. thick and 4 in. square. The centre of the hole for each honey tube was $1\frac{1}{4}$ in. from the corner. The diameter of the white circle was $1\frac{1}{2}$ in., of the blue circle one in. and of the hole for the honey tube $\frac{3}{8}$ in., approximately. The flat-bottomed glass tubes holding the honey were one in. long. They were given a very slight lip which dropped into the bevelled-out top of the hole in the centre of each flower.

Type-1B flower was made from a round Perspex plate $\frac{1}{4}$ in. thick and 7 in. in diameter. Eight flowers were painted around the plate at regular intervals

with their centres on a circle concentric to the plate and $5\frac{1}{2}$ in. in diameter. The diameter of the white circle of each flower was again $1\frac{1}{2}$ in., but the honey tube, which was stuck into the hole in the plate, had a diameter of $\frac{7}{16}$ in. and was $1\frac{1}{2}$ in. long. On each flower, eight radiating 'petals' were painted as shown in the figure.

The flowers were painted with two coats of white 'Enamel-it' lacquer and when these were dry the other colour was applied. A single, thin coat of 'Oxford blue' 'Starline' chinese lacquer was used for the blue and 'Buttercup' 'Japlae' for the yellow patterns.

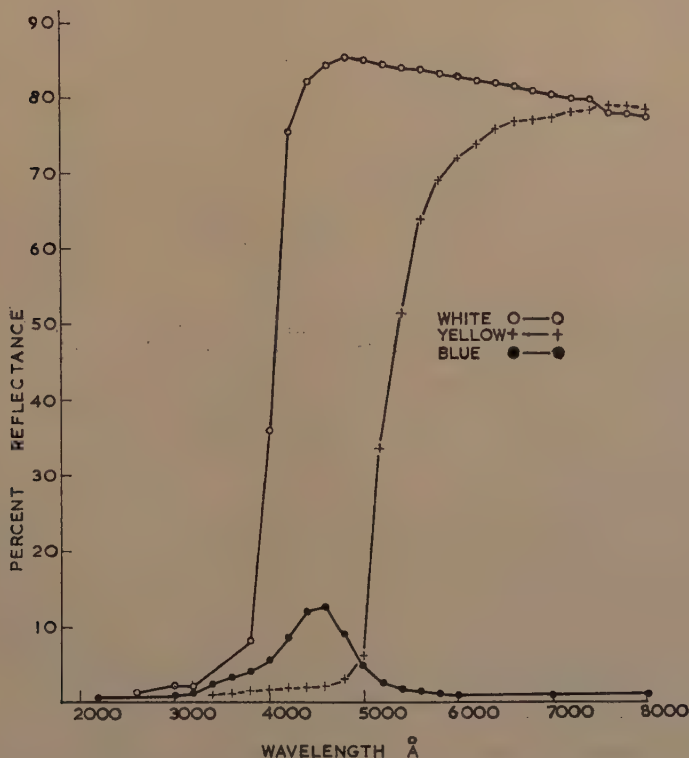


Fig. 2.—The reflectance of the three colours, white, yellow and blue, used for painting the artificial flowers as measured with a SP500 Unicam electrospectrophotometer.

The reflectance of each enamel colour painted thickly over a white under-coating on a Perspex disk was measured with a SP500 Unicam electrospectrophotometer and the results using a magnesium carbonate standard are shown in fig. 2.

When testing the responses of the insects to the flowers and the honey solution, either of the following alternatives was employed: two type-1A plates of flowers, spaced with 6 in. or 12 in. between their centres, or one type-1B plate, in which the insects have to choose between alternative flowers, whose centres are 2 in.

apart around the ring. Either method seems satisfactory, on the assumption that, in the test situation, the insects are either reacting or not reacting to one of the flower types and not making a direct comparison and then choosing between them. The two-plate method offers the insects well-spaced alternatives—an arrangement which is perhaps specially advantageous where odours are involved. There is, however, a tendency, for which it is difficult to correct, for insects to rest on certain parts of the cage so that they are nearer to one plate than the other. This biases the results unsatisfactorily so far as individual comparisons are concerned although by alternating the position of the plates at regular, short intervals during the test the final conclusions regarding preference are not affected. The single-plate method eliminates the difficulty about bias but without knowing the strength, precision and sensitivity of the insects' reaction to odour, with and without the extra stimulus of colour, it is difficult to assess the suitability of this method for tests involving odorous substances like honey.

Two methods of testing the response of the insects to the flowers have been employed. In the first method the number of insects which alighted on the flower plate with the apparent intention of feeding were counted and immediately swept off with a thin wire. For this test two type-1A flowers were usually used and their positions were interchanged at regular one-, two- or five-minute intervals. Fifty unfed insects were used and only one sex at a time since otherwise the test was interfered with by the formation of pairs. This method must be employed when the response to an empty flower is being examined, since the second method cannot be employed, but it suffers from the disadvantage that the responsiveness of the insects soon declines when they find no honey. On the other hand, if there is honey solution in some of the flowers the same hungry insects show a remarkable persistence in returning to both empty and full flowers alike. Counts of the numbers landing under these circumstances measure chiefly this persistence on the part of some individuals, and to get a clearer indication of the reaction of the total population it is necessary to catch each insect after it has alighted and begun to feed. Some tests carried out in this way are reported later.

In the second method the insects were allowed to feed on the flowers undisturbed and the volumes of the solutions taken were measured. More honey solution was added to the tubes during the day to keep the level in each tube fairly near the top and easily within reach of the insect's proboscis. The positions of the two flowers being compared were interchanged each day. At first the flowers were left in the cages for 24 hours but in later tests for only 7 hr.—between 10 a.m. and 5 p.m. In both cases a correction must be applied for evaporation and, as it is not possible to do this accurately, the latter method is preferable since with it the evaporation loss is less in relation to the volume consumed. (This is because evaporation proceeds all night but the insect only feeds during day-time.)

The difficulty in determining accurately the volume evaporated from the flowers on which the insects are feeding arises from the constantly changing level of the honey solution in the tubes. Where little solution is being drunk, the surface will be near the mouth of the tube, and the evaporation rate will be higher than from a half-empty tube on which many insects have fed. All the results of experiments on feeding have been corrected for evaporation. The values used were the volumes evaporated from tubes which were full at the beginning of the experiment.

In the course of the experiments, temperature, humidity and light intensity were measured at regular intervals, and when the tests were run for several days the number of insects surviving was determined each day.

The honey solution used in all these experiments was made by diluting Gale's 'guaranteed pure natural honey' (Joseph Farrow & Co. Ltd., Peterborough) with tap-water to give a 10 per cent. v/v solution (14% w/v). The solution was stored in a refrigerator and never used when more than seven days old. For special purposes it was made up freshly just before the test.

OBSERVATIONS ON NEWLY ESTABLISHED CULTURES.

After the original culture of *P. brassicae*, called the Cambridge culture, had been maintained in the laboratory for seven years, from 1950 until 1957, fresh larvae were obtained from Staffordshire to start a new stock. The adults which emerged from these larvae refused to feed on the artificial flowers under conditions which were exactly the same as those under which the established Cambridge stock was feeding readily. Instead, the adults behaved in a very wild manner and battered themselves against the cage as already described.

These observations suggested that the Cambridge stock had become adapted to the conditions in which the adults were caged without this fact being realised. The behaviour of the early generations of the new stock was therefore observed, to see if it also underwent a similar process of adaptation. The results obtained are shown in Table I.

TABLE I.

Showing the feeding behaviour of the adults of the first four generations of *P. brassicae* in captivity. The culture was started from eggs and larvae collected in the field.

Generation in captivity	Age of insects (days)	Feeding test day	Number of insects alive	Number observed feeding in 1 hr.	Remarks
First ..	1-3	1	50	0	The insects fed on natural flowers but not on artificial flowers except when induced to do so.
	8-10	7	4	0	
	12-14	11	2	0	
Second ..	0-2	1	50	0	The insects fed on natural flowers at all times but could not be induced to feed on artificial flowers until the afternoon of the second day. Later some fed voluntarily.
	1-3	2	50	0	
	2-4	3	48	3	
Third ..	1-3	1	50	2	No further insects fed in the following 5 hr. but later others fed. Another batch of this generation were compared with the Cambridge stock, see Table II.
Fourth ..	Batch (a) 1-2	1	50	16	The insects of this generation fed readily, and another batch survived as well as the Cambridge stock, see Table II.
	Batch (b) 3-4	1	50	38	

In the first generation no adult was observed to feed on the artificial flowers, but the cage could not be observed throughout the day and a few may have fed. These insects were, however, very ready to feed on natural flowers and could be induced to feed on the artificial flowers when held with their fore-tarsi touching the honey solution.

A sufficient number of eggs was obtained for producing the second generation. After the adults of this generation had been caged with the artificial flowers for three days, three insects were seen to feed voluntarily.

In the third generation, very many more insects fed and in the fourth generation the insects seemed to feed as well as those of the Cambridge stock (Table II).

Similar, but less detailed, observations have been made on two other new stocks. The first generation of adults in captivity in both these cases also battered themselves against the cage walls and refused to feed, but in a varying number of generations became adapted to life in the stock cage.

TABLE II.

Detailed comparison of the feeding behaviour of adults of *P. brassicae* on artificial flowers in their third and fourth captive generation with those of the old-established Cambridge stock.

Generation of new stock	Average age of insects (days)	New stock			Cambridge stock		
		Number alive (on percentage basis)	Vol. honey soln. taken		Number alive (on percentage basis)	Vol. honey soln. taken	
			ml./day	Cal. ml./100 insects		ml./day	Cal. ml./100 insects
3rd ..	1-4	100	0.7	0.7	100	3.7	3.7
	2-5	86	1.7	2.0	98	4.5	4.6
	3-6	69	1.5	2.2	93	3.4	3.7
	4-7	48	2.3	4.8	93	4.9	5.3
	5-8	41	2.2	5.4	86	6.2	7.2
	6-9	40	2.5	6.3	86	5.5	6.4
4th ..	1	100	2.8	2.8	100	3.2	3.2
	2	100	3.3	3.3	98	4.0	4.1
	3	96	5.3	5.5	96	4.5	4.7
	4	96	4.2	4.4	95	3.9	4.1
	5	92	4.8	5.2	95	4.0	4.2
	6	88	4.6	5.2	95	4.5	4.8
	10	76	—	—	70	—	—
	20	44	—	—	44	—	—
	30	8	—	—	10	—	—

OBSERVATIONS ON THE WELL-ESTABLISHED CAMBRIDGE CULTURE.

Feeding at various temperatures.

No attempt has been made to determine the exact maximum and minimum temperature beyond which fully conditioned insects will not feed. Instead, the volume of honey solution taken by insects at 20 and 30°C. has been compared over several days.

The two large-type cages in which the insects were confined were set up in adjacent, similar glasshouses. Over each cage there was the usual 500-watt tungsten filament lamp. In one cage the temperature, as measured at three levels, three times each day, fluctuated around 20°C. and in the other cage around 30°C. The relative humidity in each cage was recorded on a thermohygrograph and the light intensity was also measured twice daily. It fluctuated between 250 and >1,000 lumens/sq. ft.

Each cage was stocked with 15 males and 15 females between one and three days old and a record was kept of any that died. A standard square plate of flowers was provided in each cage and the 10 per cent. honey solution in the tubes was topped up whenever the level was seen to be low. After 24 hr. the total volume consumed was noted.

The results obtained are set out in Table III. As would be expected, very much more honey solution was consumed by the adults kept at an average

temperature of 30°C. than by those kept at an average temperature of 20°C., and insects transferred from one temperature to another immediately adjusted their consumption to the volume which was normal for the new temperature. The humidity was a little lower in the warmer cage, the insects were more active and, as will be shown in a later paper, they laid many more eggs.

TABLE III.

The average volume of honey solution taken per insect per day at 20 and 30°C. for a population started with a 50/50 sex ratio.

Treatment of insects	Average temperature of test					
	20°C.			30°C.		
	Duration of test (days)	Relative humidity (%)	Average volume taken (ml./insect/day)	Duration of test (days)	Relative humidity (%)	Average volume taken (ml./insect/day)
Kept at temperature indicated	14	44-48	0.03	14	38-45	0.07
Transferred from 20 to 30°C.	7	36-47	0.02	4	34-40	0.07
Transferred from 30 to 20°C.	4	36-47	0.01	7	34-40	0.06

Feeding in relation to the size of the cage.

The culture was originally established in the late summer of 1950 and, when the first adults emerged, it was assumed that a large cage would be more favourable for feeding and mating than a small cage, and one measuring 40 × 30 × 36 in. high which happened to be available was chosen (David & Gardiner, 1952). Subsequently the question whether a much smaller cage is unfavourable for feeding has been investigated.

Cages of two sizes, 40 × 30 × 36 in. high and 13 × 13 × 18 in. high, were set up side by side in the glasshouse so that the daylight intensity measured at the level of the artificial flowers was equal. No lamps were used above the cages and the

TABLE IV.

Comparative tests showing the average volumes of honey taken per insect per day over five-day periods in small- and large-type cages.

Period of test	Average volume of honey consumed per insect per day (ml.)			
	Experiment 1		Experiment 2	
	Large cage	Small cage	Large cage	Small cage
1st to 5th day	0.023	0.010	0.049	0.033
6th to 10th day	0.071	0.045	0.043	0.036
11th to 15th day	0.020	0.020	0.042	0.033
16th to 20th day	0.024	0.019	0.057	0.046

Illumination level 200 to 600 lumens/sq. ft.

internal temperatures, as measured at three levels, at 10 a.m., 2 p.m. and 4 p.m., were very much the same in the two cages and averaged about 25°C.

Fifteen pairs of insects 1-3 days old were used in each cage and records were kept of when insects died and of their sex. In each cage there was a square plate of standard flowers containing 10 per cent. honey solution and the volumes of solution taken were measured daily and corrected for evaporation loss.

Two experiments of this kind have been run and each lasted 20 days. The average volumes of honey consumed per insect, per day, over five-day intervals have been calculated, and these results are shown in Table IV. It can be seen that more honey was regularly consumed by the insects in the large cage than by the insects in the small cage. The difference may not always be significant but it is reasonable since the insects in the large cage might be expected to consume more energy in flight. Another explanation could be that more eggs were laid by the insects in the large cage but this is not so, as will be shown in a later paper.

Feeding on flowers at different heights in the stock cage.

As the stock cages were 36 in. high, a considerable variation was possible in the level at which the plates of flowers were placed. Usually the flowers were on stands which held them one foot from the top of the cage, and in the present series of experiments the volume of honey solution taken from flowers on this position was compared with that taken from flowers held one foot from the floor of the cage. Two plates of standard blue flowers were used in the comparison, and controls of unpainted plates screened with gauze of $\frac{1}{4}$ -inch mesh were also put in the cage so that the evaporation rate at the two levels could be measured.

Three experiments with 100 adults each were run, and it was found that during the first two days more honey solution was always taken from the upper flower than from the lower flower but after that more was often taken from the lower flower. The results of two typical experiments are shown in Table V.

TABLE V.

The volume of honey solution, corrected for evaporation, taken by 100 adults of *P. brassicae* from high- and low-level artificial flowers.

Day	Experiment 1		Experiment 2	
	Total volume (ml.) taken from Low flowers	High flowers	Total volume (ml.) taken from Low flowers	High flowers
1	0.6	1.6	0.8	1.5
2	1.2	2.5	1.3	2.7
3	1.8	2.2	2.8	1.3
4	2.4	2.1	1.7	2.6
5	2.7	2.2	2.7	1.6

It is not surprising that during the first two days more honey is taken from the higher than the lower flowers since the insects normally tend to rest in the upper part of the cage and so are nearer the high flower. Later, as the total volume of honey solution consumed goes up, the insects make more use of the lower flower. There may be various explanations for this such as that the upper flower becomes crowded and difficult to feed at, or the colour may be hidden by the feeding butterflies, or finally the insects may have learned to find the lower flower.

These results suggest that in the stock cage the flowers should be at a high level where they are more easily found by the insects during their first few days in the cage. No doubt, however, in the absence of a high flower, more insects would find their way to the low flower.

Attraction to and feeding from artificial flowers.*The response to the standard blue flowers.*

When a square plate of clean, blue and white, empty, standard flowers is placed in a cage with unfed adults 1-3 days old, many are immediately attracted. They alight on or near the blue rings and, as they do so, their proboscides may be already unrolled or they may be unrolled after landing. In either case the insect makes rapid movements with its proboscis in search of food. This observation is in agreement with the conclusion reached by Ilse (1928) that, in certain butterflies, colour alone is sufficient to induce the complete feeding reaction.

Usually when an insect finds no honey it rolls up its proboscis and flies away after a few seconds. Several visits may be paid to the flower, but after a short time the insect ceases to respond, and when a group is being tested there is a steady falling off in the number of visits paid to the empty flowers during successive time intervals.

By directly comparing the number of alightments made on empty flowers of various designs it can be shown that the insects are responding chiefly to the blue in the standard pattern. Only a few insects respond to the white ring alone, but there is a strong response to the blue ring alone (Table VI).

TABLE VI.

Comparison of the number of alightments made on the standard blue flower Type A and the component white and blue rings of this flower and on unpainted flower plates.

First flower type and sex of insects	Number alighting during time indicated	Time (min.)	Number alighting during time indicated	Second flower type
Standard flower	15	0-1	0	Unpainted
white ring with	9	1-2	0	
central blue ring	9	2-3	0	
♀	13	3-4	0	
	14	4-5	0	
	8	5-6	0	
White ring	3	0-2	0	Unpainted
without blue ring	3	2-4	0	
♀	1	4-6	0	
	5	6-8	0	
Blue ring	24	0-2	0	Unpainted
without white ring	13	2-4	0	
♀	17	4-6	0	
	12	6-8	0	
Blue ring	39	0-2	2	White ring without blue ring
without white ring	37	2-4	3	
♂				

Although no insects landed on the unpainted plates in the above experiments they have been occasionally observed to do so and also to feed when the tubes contained honey solution. In two comparative experiments run for five days with the two plates in the same cage, the average daily consumption (10 a.m. to 5 p.m.) of 10 per cent. honey solution by 100 insects from the standard flowers was 3.6 ml. and 3.7 ml. while the corresponding volumes taken from unpainted flowers were 0.8 ml. and 0.15 ml. Owing to the difficulty of making the correction

for evaporation, which has already been explained, the latter figures are only approximate and the first of them (0.8 ml.) may be high.

These results show that the main stimulus attracting caged insects to the artificial flowers is colour. The odour of fresh honey solution alone seems to exert little attraction but this subject is discussed more fully in a later section of this paper which is concerned with the honey solution.

The response to the standard yellow flowers.

In just the same way as was done for standard blue flowers, the number of alightments made on a type-1A plate of standard yellow flowers and on an unpainted plate, neither of which contained honey solution, was compared. In ten minutes, 27 insects landed on the yellow flowers and none on the unpainted plate.

An attempt was also made to compare the number of alightments made on plates of empty standard blue and on standard yellow flowers (both type-1A). At the end of 40 minutes, 145 alightments had been made on the blue flowers and 132 on the yellow flowers, suggesting that they were about equally attractive.

On the other hand, when the volume of honey solution consumed from blue and yellow flowers was compared using the type-1B flower in which, it will be remembered, the two patterns being compared alternate, it was found that more honey solution was taken from the yellow than from the blue flowers. In four experiments, each lasting five days, there was only one day out of the 20 which was an exception to this when, on the day concerned, equal volumes of solution were taken from the flowers of the two colours. The daily results are not given, but the final averages for the four experiments are shown in Table VII.

TABLE VII.

Comparison of the volume of honey solution taken in four five-day tests from type-1B blue/white and yellow/white flowers.

Date of experiment					Average volume (ml.) of honey solution taken per day, over five days, by 200 adults	
					From blue/white flowers	From yellow/white flowers
10.i.58	4.1	6.1
3.ii.58	2.5	3.7
11.ii.58	3.3	5.7
11.x.60	3.0	3.6

When, instead of being given a choice, the insects were offered honey solution from yellow flowers in one cage and from blue flowers in another cage it was found that on an average in two tests each lasting six days equal volumes of honey solution were drunk from the blue and from the yellow flowers.

The response to artificial flowers with blue rings of two different sizes.

As it is the blue in the artificial flowers which is largely responsible for their attraction, the effect of increasing the diameter of the blue ring on the attractiveness of the flower was investigated. Both alighting and feeding tests were carried out.

For the alighting test, two plates resembling the 1A type were used but the whole plate was painted white. On one plate, blue rings of the same diameter as those of the standard flower (1 in.) were painted around the four honey tubes while on the other plate the blue rings were the same diameter as the white rings

of the standard flowers ($1\frac{1}{2}$ in.). In other respects the standard test procedure was followed.

The results given in Table VIII clearly indicate that more alightments were made on the large blue rings than on the small blue rings.

TABLE VIII.

The number of alightments made by 50 male butterflies on small and on large blue rings.

Date of test	Duration of test (min.)	Number of alightments	
		small blue ring	large blue ring
7.x.60 ⁽¹⁾	12	15	27
7.x.60	14	45	85
10.x.60	8	20	64

(¹) In this test, the insects which alighted were caught as explained on p. 744.

When the volumes of honey solution taken from the tubes in the centre of the ring of each size were compared by the second method it was found, as would be expected from the result in the alightment test, that consistently more honey solution was taken from the large-ring flowers than the small-ring flowers, and this was true whether type-1A or -1B flower plates were used (Table IX).

TABLE IX.

Average volumes of honey solution taken by insects over five days from artificial flowers with 1-inch blue rings and $1\frac{1}{2}$ -inch blue rings.

Date of test	Number of insects	Type of flowers	Volume (ml.) of honey solution taken from artificial flowers with	
			small blue rings (diam. 1 in.)	large blue rings (diam. $1\frac{1}{2}$ in.)
28.iv.58	100 adults	1A	1.8	3.7
5.v.58	100 adults	1A	1.5	2.7
24.x.60	50 pairs	1B	3.5	4.7
24.x.60 (¹)	100 pairs	1A	3.1	5.2

Test temperature 24–29°C.

(¹) In this test the adults were kept in a constant-temperature room with fluorescent lights.

The honey solution and feeding.

The response of the insects to the odour of honey.

Although the full feeding response can be evoked in adults of *P. brassicae* by colour alone, this does not exclude the possibility that the insects also react to the odour of the honey solution in the artificial flowers. In view of the extraordinary sensitivity of certain insects to odours they might be expected to do so.

Two unpainted, 4-in.-square Perspex sheets of the type used in 1A flowers were placed in the large-type cage with 100 female butterflies. The temperature in the cage was approximately 28°C. and the light intensity 500 lumens/sq. ft. at the flower level. The four tubes in one plate contained fresh 10 per cent. honey solution while those in the other contained water. The number of alightments on each plate was observed over 5-minute intervals for 30 minutes. No butterfly was seen to visit either plate.

At the end of the 30 minutes the plate with water in the tubes was removed and replaced by a plate of empty standard blue flowers (type 1A). In six minutes there were 38 alightments on the plate of empty standard flowers but none on the unpainted plate with honey in the tubes. It is clear that the insects 12 to 15 in. away on the cage wall, or flying over the flowers much closer than this, are not attracted to the tubes by the odour of the honey solution.

A further experiment was conducted with a fresh batch of insects and a circular type-1B plate painted so that every other honey tube was surrounded with the blue and white rings of the 1A flowers while the intervening tubes were left plain. One of the painted flowers and all of the unpainted flower tubes contained 10 per cent. honey solution and the other three tubes with painted 'corollas' were empty. When a plate of this type was exposed in the stock cage no insects alighted in the vicinity of any of the three honey tubes not surrounded by blue and white, nine insects alighted on the blue and white flower filled with honey solution and 10, 11 and 11 insects, respectively, alighted on the three empty blue and white flowers.

It is clear that the reaction of the insects was dominated by their response to the blue colour and they apparently showed no response to the odour of the honey solution since even when they passed very close to the tubes, brought there by the attraction of the adjacent blue flowers, they were not diverted to these tubes even though they were hungry and three of the four blue flowers were empty.

Although the insects did not respond to the odour of honey in tubes without painted 'corollas', it might still be expected that under the stimulus of colour the responsiveness of the insects to odour would be increased, since the feeding reaction is ultimately only fulfilled when honey is found. And so, that when the response to colour had brought the insect into the vicinity of food, the response to odour would take over and direct the insect to its goal. In the case of a bee, for example, it has been shown that she often only discerns the odour when she is within an inch or so of the flower (Butler, 1951).

The experiments reported above do not support these ideas as far as *P. brassicae* is concerned, but on the other hand they do not finally prove that the insects never do react to the odour of honey in the artificial flowers. Indeed, by choosing the right conditions it can be shown that they do.

TABLE X.

Comparison of the number of alightments of 50 adults of *P. brassicae* on two plates of type-1A artificial flowers which were either empty or contained 10 per cent. honey solution or water.

Date of test	Test procedure (1)	Colour of flowers	Sex of insects	Duration of test (min.)	Number of alightments on painted flower when tube contains		
					Honey	Water	Nothing
20.xi.58 ..	A	Blue	♂ + ♀	30	163	—	41
21.xi.58 ..	A	Blue	♂ + ♀	30	192	—	70
7.x.60 ..	A	Yellow	♂ + ♀	12	116	—	90
7.x.60 ..	A	Yellow	♂ + ♀	12	143	105	—
20.x.60 ..	A	Yellow	♂ + ♀	12	84	63	—
20.x.60 ..	B	Yellow	♂ + ♀	24	15	9	—
21.x.60 ..	B	Yellow	♂	12	15	4	—

Insects 2-3 days old, test temperature within the range 24-28°C., illumination level within the range 300 to 400 lumens/sq. ft.

(1) (A) Insects merely driven off when they alighted so that the same insects could alight repeatedly. (B) Insects caught when they alighted and removed from the cage.

Except in one case, the experimental situations so far described in this section did not test the capacity of the insects to detect the odour of honey in the presence of colour. When this was done more carefully, the insects being given a choice between colour *plus* honey odour and colour *plus* an empty tube or a tube containing water, it was found that more visits were paid to the artificial flowers containing honey solution than to those which were empty or contained only water. The results of several variations of this type of experiment, all of which lead to the same conclusion, are shown in Table X. The two plates of flowers being compared were left in the same positions throughout the first test (20.xi.58) and then reversed for the second test, in which the same insects were used (21.xi.58). In all the other tests the positions of the flowers were interchanged at intervals of either 2 or 3 minutes for the reasons given on p. 744.

It can be concluded from the experiments in this section that adults of *P. brassicae* show no response to the odour of honey alone, under the test conditions described, even when brought near to the source of the odour by the attraction of adjacent coloured flowers. When, however, honey odour is associated with colour in artificial flowers they react to these flowers in preference to those which do not contain honey.

The influence of the concentration of the honey solution on the volume consumed.

Wild adults of *P. brassicae* feed on nectar, and honey was the obvious choice of food to put in the artificial flowers. It was decided, quite arbitrarily, when the culture was established, that a 10 per cent. (v/v) solution should be satisfactory but, subsequently, experiments have also been made with solutions of 1 and 20 per cent.

In the first series of experiments, the insects were given the choice between two concentrations of honey solution contained in separate type-1A plates of artificial flowers. The plates of flowers were between 6 and 12 in. apart and their positions were interchanged daily. At the beginning of the various tests the insects were between 1 and 5 days old, the temperature in the test cages varied from about 22 to 28°C. at different times and the light intensity between 200 and 800 lumens/sq. ft.

From the results given in Table XI it can be seen that the insects took very little 1 per cent. honey solution when 10 per cent. was available as an alternative choice, and that they showed a less well marked preference for 20 per cent. solution compared with 10 per cent. solution.

TABLE XI.

Comparison of the volumes of 1 per cent. and 10 per cent. and of 10 per cent. and 20 per cent. honey solution taken by adults of *P. brassicae* (50 ♂♂ and 50 ♀♀) given a choice between the two in one cage.

Date of test	Duration of test (days)	Average volume (ml.) of honey solution consumed per day by 100 insects		
		1% v/v	10% v/v	20% v/v
17.iii.56	2	0.2	6.7	—
22.x.57	5	0.9	5.3	—
28.x.57	3	0.2	4.0	—
15.viii.56	2	—	2.6	3.8
17.iv.57	5	—	0.9	1.2
9.iii.59	5	—	1.8	3.3

When the insects were offered the alternative concentrations of honey solution in two adjacent cages under similar conditions it was found that the insects offered 10 per cent. solution drank more during the first days of the test than those on the 1 per cent. solution, but later the reverse was the case. Taking into account the results given in Table XI, this probably means that at first the insects, offered the relatively unacceptable 1 per cent. solution, rejected it and depended on their reserves. Later, to meet the need for food, for energy and in the case of the females for yolk formation, they imbibed a larger quantity of the 1 per cent. solution than did those on the 10 per cent. solution in an effort to ingest an adequate amount of nutriment. When the 10 and 20 per cent. honey solutions were compared, more of the 10 per cent. solution than of the 20 per cent. solution was taken.

The average results obtained in two experiments with 1 and 10 per cent. honey solutions and of two experiments with 10 and 20 per cent. honey solutions are shown in fig. 3 (A and B).

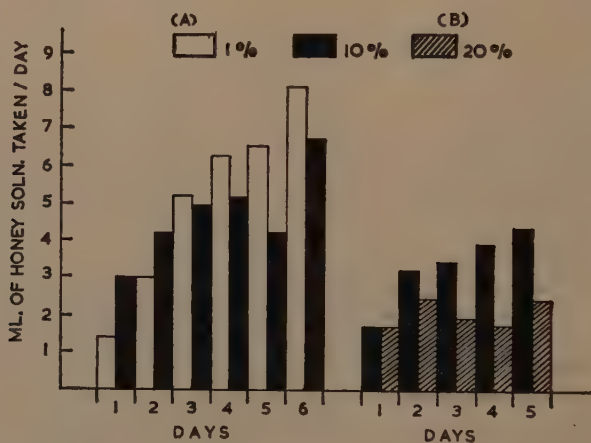


Fig. 3 (A and B).—The volumes of (A) 1 and 10 per cent. and (B) 10 and 20 per cent. honey solutions taken in successive days by 100 adults of *P. brassicae* offered the alternative concentrations in separate cages.

The influence of the concentration of the honey solution on the survival of the cultures.

Nectar, the normal food of the adults, has an average water content of about 60 per cent. for a typical sample, but this value varies greatly with the plant from which it is taken, the weather and a variety of other factors. In addition to feeding on nectar, the adults are known to drink water.

A typical sample of honey, on the other hand, contains only about 16 to 20 per cent. weight of water, and this figure is fairly constant (Eckert & Allinger, 1939; Pryce-Jones, 1944). Since honey is more concentrated than nectar, and water is taken in addition to nectar it was considered that it would be necessary to dilute the honey to provide, in one solution, a fairly balanced intake of food and water.

As already stated, a 10 per cent. (v/v) (14 per cent. wt./vol.) solution was decided upon quite arbitrarily, but some cultures of *P. brassicae* have also been maintained using 1 per cent. (v/v) honey solution. When the length of life of the adults in the two cultures was compared it was found that the insects survived

for a shorter time on 1 per cent. than on 10 per cent. honey solution, and also that they laid only about one-tenth as many eggs during their life. The percentage survival of males and females up to the 15th day is shown separately in fig. 4 and the egg-production will be discussed in detail in a later paper.

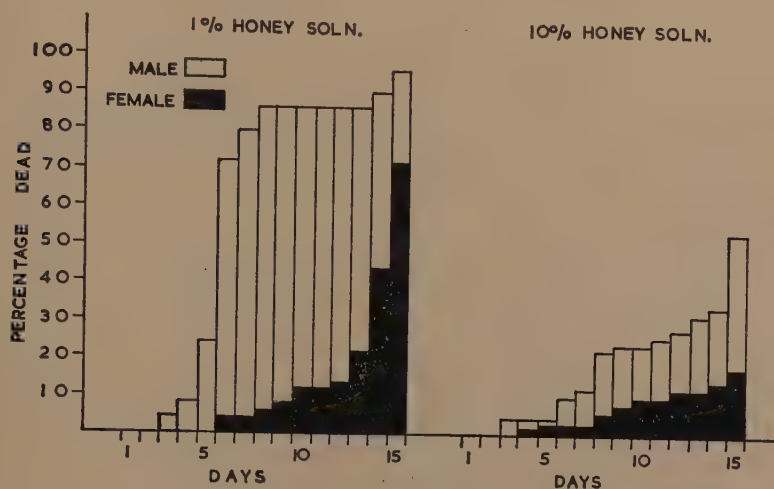


Fig. 4.—The survival of adults of *P. brassicae*, 2-3 days old, given either 1 or 10 per cent. honey solution under normal glasshouse conditions. Temperature, 20 to 25°C.

The influence of the freshness of the honey solution on the volume consumed.

The honey solution used to feed the insects was commonly made up about twice a week and stored in the refrigerator. This remained fresh, but the honey left over in the flowers was not poured away each day so that they usually contained a certain proportion of solution which had begun to ferment. It seemed possible that fermentation might influence both the attractiveness of the honey solution and also its palatability.

TABLE XII.

Comparative tests showing the volume of fresh and stale 10 per cent. honey solution taken by adults of *P. brassicae* from standard flowers.

Date of test	Duration of test (days)	Temperature at which honey solution was stored (°C.)	Average volume (ml.) of honey solution taken per day by 100 insects			
			Fresh	2 days old	4-5 days old	21 days old
18.vi.56	2	25	5.4	5.6	—	—
26.viii.56	2	25	4.1	3.3	—	—
16.x.58	6	17-24	2.5	2.9	—	—
28.viii.56	2	25	10.0	—	3.4	—
17.x.56	6	16-25	4.4	—	3.0	—
17.iv.57	2	25	6.3	—	—	1.4

The two solutions were offered in the same cage except in the last test when the fresh and the 21-day-old honey were offered in separate cages.

In several comparative experiments, details of which are given in Table XII, it was found that less stale honey solution than fresh honey solution was taken by the insects. The effect did not become very evident in these tests until the honey solution was four to five days old. Honey solution three weeks old was quite unsuitable. After two days, 95 per cent. of the insects feeding upon it were dead, whereas only 5 per cent. of the insects given fresh honey died in this time.

Comparison of honey solution and sucrose solution as food for adults of P. brassicae.

When the culture of *P. brassicae* was originally established it was felt that if honey solution was used in the artificial flowers its odour would assist the insects in finding them. When later it was found that the response of the insects to colour alone was quite sufficient to bring them to the artificial flowers, and to evoke the full feeding reaction, it seemed unnecessary to use honey, and sucrose was considered as an alternative.

Unlike pure sucrose, however, honey contains small quantities of many substances which may make it a more balanced and complete food for the insects. Analysis has shown that, in addition to sugars, a typical honey contains gums, tannins, dextrans, essential oils, esters, mineral salts, acids, yeasts, proteins, enzymes and traces of vitamins (Pryce-Jones, 1944). The average ash content was found to be 0.326 per cent. and the total 'undetermined' content, in which most of the above substances except dextrin were included, was 4.87 per cent. (Eckert & Allinger, 1939). The protein content is usually reported to be low, and a value of 0.2 per cent. has been given (Butler, 1954). Haydak & others (1942) report that they found six vitamins in honey—thiamin (B_1), riboflavin (B_2), pyridoxine (B_6), ascorbic acid (C), pantothenic acid and nicotinic acid. The amounts present were variable. Some of the total vitamin was dissolved in the honey but the remainder was associated with pollen grains.

It is perhaps unlikely that adults of *P. brassicae* could make any use of the protein, since no protease has been found in the digestive system of adult Lepidoptera (Wigglesworth, 1953) but it seemed possible that the mineral salts would be used and vitamins of the B group are known to be required by insects (Wigglesworth, 1953).

To determine whether sucrose solution was as satisfactory as honey solution for feeding the adults, two cultures, each containing 50 males and 50 females, were maintained side by side in the glasshouse. To ensure that the conditions in the two cages were alike, their positions were interchanged daily. One culture was given pure, fresh 11.2 per cent. w/v sucrose solution as its sole food and the

TABLE XIII.

A comparison of the survival of males and females of *P. brassicae* fed on honey solution and sucrose solution.

Observation	Experiment 1		Experiment 2	
	Honey	Sugar	Honey	Sugar
Average volume (ml.) of solution consumed per 100 insects per day during the first six days ..	4.3	3.8	7.2	7.9
Number of insects surviving on day 14 ♂	34	36	36	32
.. .. . ♀	42	41	41	32
Number of insects surviving on day 20 ♂	29	27	22	17
.. .. . ♀	35	26	28	14

other the normal 10 per cent. (v/v) honey solution which contained about 11.5 per cent. w/v of total solids, of which about 1.5 per cent. w/v consisted of the substances other than sugar, listed above, which occur in honey. The solutions were made up in distilled water.

The insects in the two cages were kept well supplied with fresh honey or fresh sucrose solution, and a plant was provided on which the females could lay, and the number of males and females which had died were noted daily. For the first six days, records were kept of the volumes of sugar and honey solution drunk in each cage.

Two experiments, each lasting 20 days, were carried out in this way, and the results obtained are given in a condensed form in Table XIII.

It can be seen that females feeding on honey solution survived substantially longer than those feeding on pure sucrose solution. The difference in survival only developed after the 14th day in the first experiment but was already apparent by this time in the second experiment. There is also some evidence from the second experiment that males survived less well on sugar than on honey, but this conclusion is much less definite.

These results are entirely in accordance with what might be expected if, in fact, the honey solution supplied valuable ingredients lacking in the sucrose solution. Thus the females which provide the material for the eggs laid each day might be expected to suffer from any nutritional deficiency more acutely than the males, and furthermore that this deficiency would only become crucial when a large part of the body reserves have been drawn upon to supply the materials, other than those derived from sugar, required for the eggs.

In practice, however, it may be concluded that, except for the fact that the females will not live so long, sucrose solution would be as suitable as honey solution for maintaining a culture.

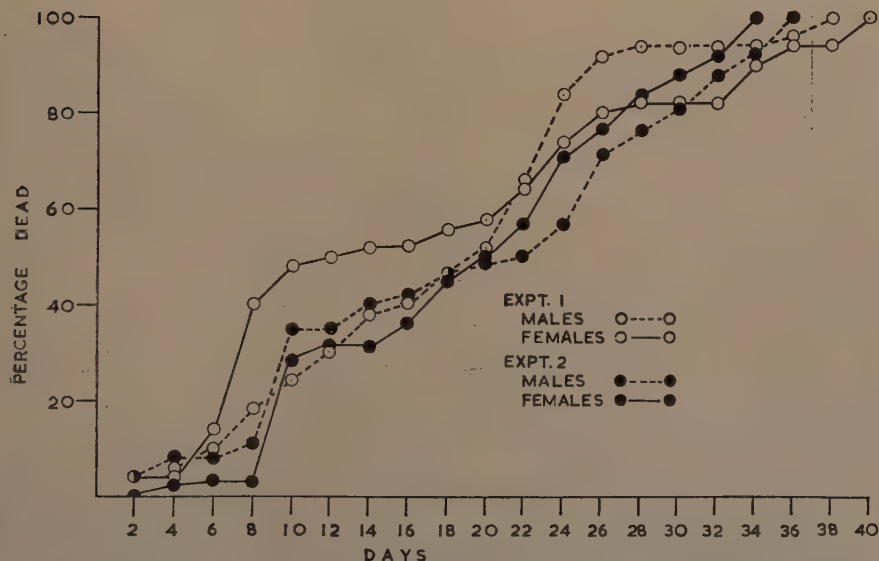


Fig. 5.—The survival of adults of *P. brassicae*, 2–3 days old, fed on 10 per cent. honey solution in two typical cultures maintained under glasshouse conditions as described in the text.

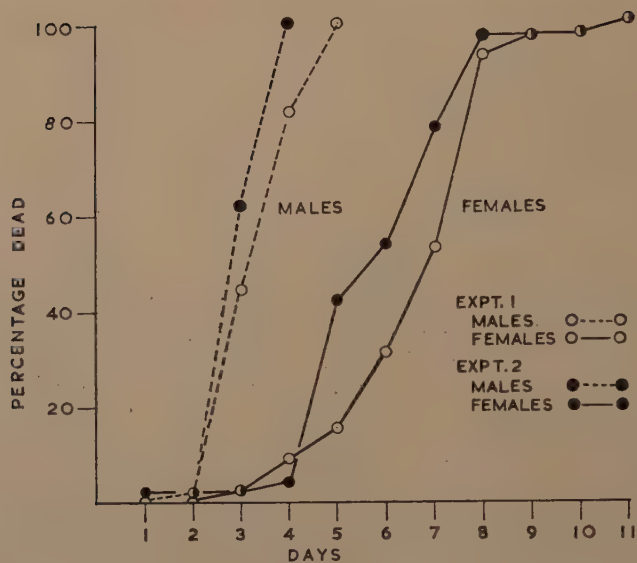


Fig. 6.—The survival of starving adults of *P. brassicae* in two typical cultures maintained under glasshouse conditions as described in the text.

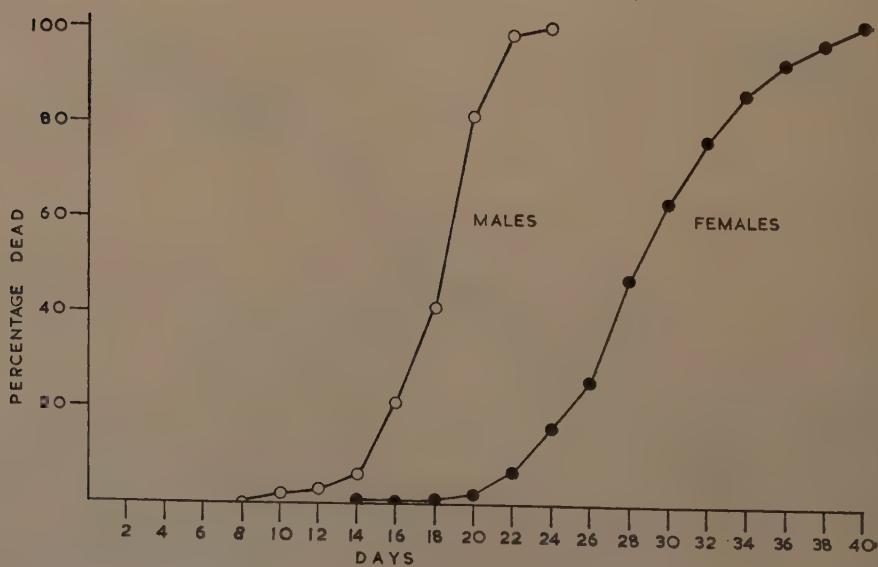


Fig. 7.—The average survival of starving males and of starving females in three typical cultures kept in an air-conditioned room at 12.5°C. and 60 per cent. relative humidity.

Length of life of the adults when fed and starving.

Observations have been made to determine how long the adults live in the stock cage under normal glasshouse conditions. Some typical results obtained with adults taken from the stock culture are shown in fig. 5. In the experiments, the insects emerged in a constant-temperature room at 25°C. and 60 per cent. relative humidity and were placed in the stock cage when 1 to 2 days old. They were given 10 per cent. (v/v) honey solution in standard flowers and potted cabbage plants on which to oviposit. The temperature, light intensity and humidity in the greenhouse fluctuated considerably during the tests and, taking into account night and day levels, it was found that the temperature varied between 15 and 30°C., the relative humidity between 30 and 55 per cent. and the illumination level from near nought at night to over 1,000 lumens/sq. ft. during a sunny day.

As would be expected, there is some variation between batches but it appears that about half the insects survive for 18 days but very few, if any, survive for longer than 36 days. Males or females may live the longer.

When similar tests were carried out on starving insects held in large cages under the same glasshouse conditions all the insects had died by the 11th day and the males survived for a shorter time than the females (fig. 6).

Although starving insects very soon die under glasshouse conditions they can be stored for much longer if kept at 12.5°C. and 60 per cent. relative humidity. Starting with insects 1-2 days old, under these circumstances half the males survive for about 17 to 19 days but all are dead by about the 23rd day. Half the females survive for about 28 to 30 days but all are dead just before the 40th day. The average results of three experiments are shown in fig. 7.

Discussion and conclusions.

The experiments on the feeding behaviour of *P. brassicae* described in this paper form part of an investigation into the conditions which affect the productivity of a laboratory culture. Those influencing mating have already been discussed (David & Gardiner, 1961).

The very important process of adaptation to feeding on artificial flowers, which takes place in all newly established stocks of *P. brassicae* during the first few generations, has been considered in the introduction to this paper. As it only occurs in this first period of the life of a culture it is rather easy to overlook its true significance and to assume afterwards that difficulties encountered at this stage were due to inexperience. That this is not so was proved anew at each attempt to establish a fresh culture from eggs or larvae collected in the field: most of the resulting adults ignored the artificial flowers.

This process of adaptation is interesting in itself but the observation embodies a principle of much wider application to the establishment of laboratory cultures of insects—namely that from a wild stock, which is at first very difficult to rear in captivity, a strain may be derived which lives and multiplies quite freely.

Some instances of the adaptation of insects to breeding in captivity have been found in the literature. The silkworm, *Bombyx mori* (L.), is certainly the oldest and the most extreme example—it no longer occurs except in artificial culture. Early workers failed to maintain body lice, *Pediculus humanus humanus* L. (*P. h. corporis* Deg.), for more than two generations on rabbits, but Culpepper (1946) was able to feed a normal colony for six generations on certain rabbits and this technique is now used for maintaining a large stock culture. It is interesting that Smith & Eddy (1954) describe newly caught lice stocks as 'wild' in their behaviour; they are restless and do not settle and feed as well as lice from established laboratory stocks. The behaviour of the first generation of adults of *Pieris brassicae* bred from eggs or larvae collected in the field has been described in the introduction to this paper and might also very aptly be termed 'wild' in

comparison with that of later generations. And like lice they, too, are reluctant to feed.

More recently Stahler (1959) has described selective changes in a colony of *Anopheles quadrimaculatus* Say maintained in the laboratory for seven years. The percentage of females inseminated was significantly higher during the years 1953-55 than during 1950-52, when sexual activity was low during the winter months. In subsequent years the phase of low sexual activity in winter disappeared and it is thought that the improved survival of males which occurred at the same time was responsible.

The question arises why it is that most of the first generation of adults fail to feed on the artificial flowers and what is the nature of the change that leads to the production of a strain which feeds readily.

At the present time there is very little evidence to go on and there seem to be a variety of possibilities. For example, the few insects in the early generations which do feed may do so because (a) they find the flowers by chance and learn to revisit them, (b) they are more stimulated by the particular blue or yellow used on the flowers than are the other insects, and (c) they are less obsessed with the urge to escape, less wild that is, and being less under the influence of the urge they respond more readily to the urge to feed.

It is evident that during these early generations a strong selection is exerted in favour of any insects which do feed and this may be the full explanation of the development of the stock which feeds readily. It is, however, possible that some other factors contribute. For example, in the culture, the larvae are reared under much more crowded conditions than they would encounter in the wild and this may affect the behaviour of the resulting adults.

An effect of this kind has been noted with the western tent caterpillar, *Malacosoma pluviale* (Dyar). Active and inactive larvae of this insect have been described, reduction of food quality and quantity exaggerates any innate sluggishness and may even lead to an increase in numbers of the sluggish larvae in the next generation. The sluggish larvae give rise to sluggish adults, whereas the active larvae give adults which batter their wings against the walls of containers (Wellington, 1957).

There is another factor which may influence the feeding response: when the majority of a stock are of a strain which feeds readily they will congregate on the flowers and attract non-feeders which, after a taste of honey, learn to find the flowers of their own accord.

Summary.

Only a few individuals in the first generation of adults of *Picris brassicae* (L.) reared in captivity will feed on artificially prepared flowers. By breeding from the eggs laid by these individuals and from a few others which have been helped to feed it is possible to maintain the stock. In each successive generation a few more individuals feed, and after about four generations most of the insects feed voluntarily. Finally, after many generations, all the insects use the flowers, and the experiments described are concerned with this adapted stock.

Many factors influence the attraction of the insects to the artificial flowers and the volume of food taken. Colour, blue or yellow, is responsible for attracting the adults to the flowers, and it is only when they are very close to the flowers and under the stimulus of colour that they show any detectable reaction to the odour of the honey solution in the flowers. Larger blue flowers are more attractive than smaller blue flowers.

The insects find the artificial flowers more easily, at first, if they are high in the cage. More honey solution is taken at 30 than at 20°C. and more in a large cage than in a small cage. When given a choice, the insects take more 10 per cent. (v/v) than 1 per cent. (v/v) honey solution and more 20 per cent. (v/v)

solution than 10 per cent. (v/v) solution. When offered two of these solutions in separate cages the insects at first take less of the 1 per cent. solution than of the 10 per cent. solution. Later, presumably in an effort to obtain sufficient nutriment, they take more of the weaker solution than of the stronger solution. The insects live for a much shorter time on the 1 per cent. solution than on the 10 per cent. solution. Fresh honey solution is preferred to a solution 4-5 days old, and older honey solution is quite unsuitable as food. When the survival of adults fed on honey and sucrose solution is compared it is found that females, especially, survive longer when feeding on honey.

About 50 per cent. of the adults fed on 10 per cent. honey solution in the normal stock cage under glasshouse conditions live for 18 days. Very few survive for longer than 36 days. Starved insects all die by the 11th day under similar conditions. When starved at 12.5°C. and 60 per cent. relative humidity, about half the males live for 17 to 19 days, but all are dead by about the 23rd day, whereas half the females live for about 29 days and some survive up to 40 days.

Acknowledgements.

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THE SUSCEPTIBILITY OF TSETSE FLIES TO TOPICAL APPLICATIONS
OF INSECTICIDES. II.—YOUNG ADULTS OF *GLOSSINA MORSITANS*
WESTW. AND ORGANOPHOSPHORUS COMPOUNDS,
PYRETHRINS AND SEVIN.

By G. F. BURNETT

*Tropical Pesticides Research Institute,
Arusha, Tanganyika.*

This work continues that reported in an earlier paper (Burnett, 1961), which was concerned with the susceptibility of *Glossina morsitans* Westw. to chlorinated hydrocarbon insecticides. The purpose has been to evaluate a number of insecticides of other classes, less in the hope of finding one better than the extremely effective Telodrin*, than to have useful information available should tsetse flies acquire resistance to the chlorinated hydrocarbons. None of the compounds reported on here has been used in practical control work as far as is known to the author.

Methods and materials.

The methods and materials, including the test insects, were the same as those described in the paper cited above except that different solvents had to be used. Several organophosphorus compounds were sufficiently miscible with kerosene but others were not and it was thought desirable to use the same solvent for all compounds of this class. Only a limited range of possible solvents was readily available, mainly alcohols which gave high control mortalities. Toluene was quite innocuous but too mobile to be used easily in the Kerr microburette (Kerr, 1954). It was however the only solvent suitable for Sevin, which was applied in toluene solution. Organophosphates were eventually used in solution in decalin (decahydronaphthalene), which was found to be close to lighting kerosene in the necessary properties. Pyrethrins and pyrethrins synergised with piperonyl butoxide were dissolved in lighting kerosene, both the concentrate and the dilute solutions being kept in the refrigerator. Preliminary tests showed that mortalities increased, although not rapidly, as the proportion of piperonyl butoxide was raised from 5 to

TABLE I.

Mortality percentages, corrected for control deaths, of young adult males of *G. morsitans* treated with given insecticides. 72-hour mortalities.

Insecticide	No. flies used	Dates	Dose per fly (μ g.)					
			0.0017	0.0031	0.0068	0.0135	0.054	0.108
Malathion ..	50	14-16.x.60	—	—	—	42	—	23
Methyl-parathion	29	14.x.60	—	—	—	33	—	100
DDVP ..	40	21.xii.60	0	—	11	56	—	—
Muscatox ..	22	21-23.xii.60	—	8	—	100	—	—
Sevin ..	15	21.x.60	—	—	—	—	15	—

* 1,3,4,5,6,7,8,8-octachloro-3a,4,7,7a-tetrahydro-4,7-methanonaphthalan.

TABLE II.

Mortality percentages, corrected for control deaths, of young adults of *G. morsitans* treated with diazinon and Baytex in decalin solution. 72-hour mortalities.

Insecticide	No. of flies	Sex	Mean wt. (mg.)	Dates	Dose per fly (μ g.)								
					0.0017	0.0034	0.0068	0.0101	0.0135	0.027	0.054	0.108	Control
Diazinon	122	♂	24.1 \pm 2.25	} 21.xi.60- 9.ii.61	—	—	24	33	53	93	—	—	4
	169	♀	29.1 \pm 3.7		—	—	0	15	43	87	100	—	7
Baytex	139	♂	23.4 \pm 3.0	} 10.i.61- 14.ii.61	6.5	36	68	—	93	—	—	—	5
	109	♀	29.6 \pm 2.8		—	26	52	—	100	—	—	—	5

TABLE III.

Mortality percentages, corrected for controls deaths, of young adults of *G. morsitans* treated with pyrethrins and synergised pyrethrins* in kerosene solution. 24- and 48-hour mortalities, respectively.

Insecticide	No. of flies	Sex	Mean weight (mg.)	Dates	Dose per fly (μ g.)									
					0.00042	0.00063	0.00084	0.00126	0.0017	0.0021	0.0025	0.0030	0.0034	Control
Pyrethrins	174	♂	28.9 \pm 3.7	i.60	—	—	—	—	34	51	86	76	84	0
	138	♀	32.4 \pm 4.0	i.60	—	—	—	—	44	50	79	81	87	0
Synergised pyrethrins	131	♂	23.2 \pm 2.90	} 11.ii.- 9.iii.61	15	39	42	58	93	—	—	—	—	0
	78	♀	29.4 \pm 2.89		—	16	30	50	74	—	—	—	—	0

* Pyrethrins + piperonyl butoxide, 1:15.

10 and thence to 15 parts per part of pyrethrin and the 15:1 ratio was selected for test. Pyrethrin was evaluated in early 1960 but the synergised product was not utilised until late 1961, when a check with males at a single dosage showed no change with plain pyrethrins from the previous year. A constant volume of 0.0216 microlitres (μ l.) were used, as before, for each topical application.

The purpose of the investigation was, as stated above, to evaluate insecticides of new classes, but the difficulty experienced in getting supplies of tsetse flies made it essential to concentrate on the most effective. The investigation (apart from pyrethrins) was started by testing candidate compounds with small numbers of male flies at fairly high dosages. The following compounds were included: malathion, methyl-parathion, diazinon (O,O-diethyl O-2-isopropyl-4-methyl-6-pyrimidinyl phosphorothioate), Baytex (O,O-dimethyl-O-3-methyl-4-methylthiophenyl phosphorothioate, also known as fenthion), DDVP (dimethyl 2,2-dichlorovinyl phosphate, also known as dichlorvos), Muscatox (O,O-diethyl O-3-chloro-4-methyl-7-coumarinyl phosphorothioate, also known as coumaphos), and Sevin (1-naphthyl N-methylcarbamate).

Solutions containing pyrethrins were made by diluting fresh concentrate of certified concentration (26.1 per cent. w/w). The organophosphorus compounds were supplied as technical products of stated purity by their manufacturers or agents and Sevin was extracted and recrystallised from a 90 per cent. wettable powder, the pure compound not being obtainable in time for the completion of the investigation. Mortalities were taken at 24 hours for pyrethrins, 48 hours for synergised pyrethrins and 72 hours for the other compounds.

In the preliminary tests, only diazinon, Baytex and Muscatox gave 100 per cent. mortalities with 0.027 μ g. or less per fly and the others were not considered further. Muscatox is a difficult substance to dissolve and was discarded for that reason but diazinon and Baytex were investigated fully.

Results.

The mortalities obtained with those insecticides that did not pass the preliminary screening are given in Table I. Results for diazinon and Baytex, and for pyrethrins, are given in Tables II and III, respectively. These have been plotted in fig. 1 and regression lines constructed and tested by the methods of Litchfield & Wilcoxon (1949), with the results listed in Table IV.

TABLE IV.

Dosage-mortality constants for young adults of *G. morsitans* (data of Tables I & II).

Insecticide	Sex	LD50 (μ g.)	95% fiducial limits of LD50 (μ g.)	Ratio of LD50's		LD95 (μ g.)	Ratio of LD95's for in- secti- cides
				for sexes	for in- secticides		
Diazinon ..	♂	0.0115	0.0094—0.014	1.4 (sign.)	♂ 2.4 (sign.)	0.033	♂ 2.1
	♀	0.0163	0.013 —0.0195		♀ 2.9 (sign.)	0.034	
Baytex ..	♂	0.0048	0.004 —0.006	1.2 (not sign.)	♂ 2.6 (sign.)	0.016	♀ 2.3
	♀	0.0056	0.004 —0.007		♀ 1.6 (sign.)	0.015	
Pyrethrins	♂	0.0020	0.0018—0.0022	(not sign.)	♂ 2.6 (sign.)	0.004	♂ 1.5
	♀	0.00195	0.0017—0.0023		♀ 1.6 (sign.)	0.004	
Synergised pyrethrins	♂	0.00085	0.00073—0.001	1.4 (sign.)	♂ 2.6 (sign.)	0.0026	♀ 1.2
	♀	0.0012	0.00096—0.0015		♀ 1.6 (sign.)	0.0035	

The organophosphates killed slowly, and even DDVP, which is claimed by the manufacturers to be very rapid in action, gave kills which increased up to 72 hours. At the lower dosages used, Baytex gave very little mortality for the first 48 hours. Pyrethrins, of course, killed rapidly but the addition of piperonyl butoxide slowed the action, particularly at high dosages when the actual amount of the synergist was greatest. Hewlett (1960) reports that this effect is common and due to the retention of the pyrethrins in solution in the synergist.

Baytex was the most lethal organophosphate tested and although slightly more toxic than γ BHC the difference is not significant. Both dieldrin and Telodrin are significantly more toxic than Baytex (2.8 and 7.7 times, respectively); in all cases comparison is made with the LD₅₀'s found for the less susceptible batch in the

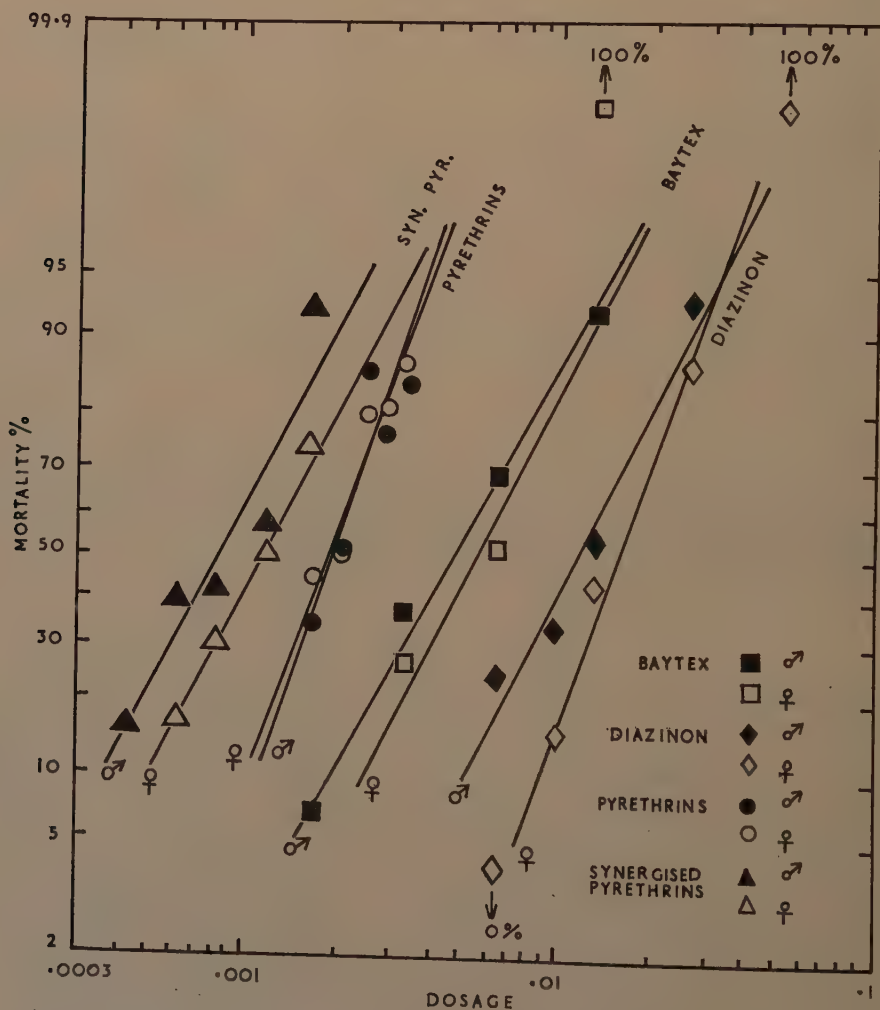


Fig. 1.—Log-dosage/probit-mortality regression lines for young adults of *G. morsitans* in respect of diazinon, Baytex, pyrethrins and pyrethrins synergised with piperonyl butoxide (data from Tables II and III). Abscissae: dosages (μg), plotted on logarithmic scale. Ordinates: mortality percentages, plotted on a linear scale of probits.

earlier tests (Burnett, 1961). Diazinon is about equitoxic to DDT but the regression lines are not parallel. It is unusual in giving significantly different LD50's for the sexes, but the lines for males and females cross at about LD95.

The addition of piperonyl butoxide to pyrethrins not only reduced the median lethal dose but caused a significant difference between the sexes. The reduction in LD50, however, is unusually small (*cf.* Hewlett, 1960) and although the lines are parallel statistically they in fact converge at high mortalities (fig. 1) and the ratio of LD95's is only 1.2 and 1.5 times, for females and males, respectively.

Discussion.

The slopes of the regression lines for diazinon, Baytex, and pyrethrins are all steeper than those previously obtained for chlorinated hydrocarbons; it has frequently been noted for other insects that compounds of the latter class tend to have shallow slopes. There also seems a tendency with organophosphates for the line for the females to be steeper than that for the males, so that the difference in LD95 is negligible although the LD50's may differ considerably. In addition to the two examples given here, this has been noted with wild-caught examples of *G. swynnertoni* Aust. and especially with old examples of *G. morsitans* (results to be published). The effect of the synergist on the slope of the pyrethrin lines is unusual (Hewlett, 1960) and the fact that it occurs in both sexes independently although the toxicity level is different (fig. 1) suggests that the effect is real and might reach significance with larger samples of flies. For practical purposes the convergence of the lines means that synergising with piperonyl butoxide will be of no practical use as a means of economising in the widespread utilisation of pyrethrins for freeing vehicles and pedestrians from tsetse.

None of the compounds reported on can compete with the more active chlorinated hydrocarbons if toxicity to young flies is taken as a base for comparison. Pyrethrum is far too expensive for further consideration except for special cases—the cost per lb. of active material is some twenty times that of dieldrin. Baytex is a good deal less toxic than dieldrin or Telodrin to young flies but very useful to have available should tsetse acquire resistance to chlorinated hydrocarbons. It is even possible that its price might fall sufficiently to compete with dieldrin on a cost basis, providing suitable cheap solvents can be found. It is useful to know that malathion and methyl-parathion, which are readily available, extensively used and relatively cheap, are unfortunately ineffective.

Summary.

Solutions of six organophosphorus compounds, Sevin, and pyrethrins (alone or synergised with piperonyl butoxide) were applied by microburette in drops of constant volume (0.0216 μ l.) to the dorsum of the thorax of young adults of *Glossina morsitans* Westw., 2–5 days old that had taken their first blood-meal the previous day. The solvents used were decalin (decahydronaphthalene), toluene and lighting kerosene, respectively.

Malathion, methyl-parathion, DDVP (dichlorvos) and Sevin were eliminated in preliminary tests as insufficiently toxic. Muscatox (coumaphos) was reasonably toxic but not readily soluble and was therefore not considered further. Diazinon and Baytex (fenthion) were fully evaluated; the former was about as lethal as DDT, the latter as γ BHC (LD50 about 0.004 μ g.). The LD50 of diazinon for males (0.0115 μ g.) was significantly smaller than that for females (0.016 μ g.) but the LD95 was much the same for both sexes.

Pyrethrins were about equitoxic with dieldrin (LD50, 0.002 μ g.); when synergised with 15 parts of piperonyl butoxide to one of pyrethrins the LD50 for males was reduced to less than half this value, and there was a significant difference in susceptibility of the sexes, the LD50 for females being 1.4 times that for males.

However, the slopes of the regression lines were such that at LD95 the difference between synergised and plain pyrethrins was too small to be of any practical use.

These results show that, judged by innate toxicity to young flies, none of these insecticides can compete with dieldrin or Telodrin for practical control, although Baytex is a useful reserve should *Glossina* acquire resistance to chlorinated hydrocarbons.

Acknowledgements.

I am grateful to the several manufacturers for the supply of samples of insecticides, to Dr. J. Ward, who suggested the trial of a synergist for pyrethrins, and to Mr. E. T. Mesmer, who extracted a purified sample of Sevin from the wettable powder. The work was part of a programme approved by the Colonial Pesticides Research Committee and financed from funds at their disposal and carried out under the Director, Tropical Pesticides Research Institute, Arusha.

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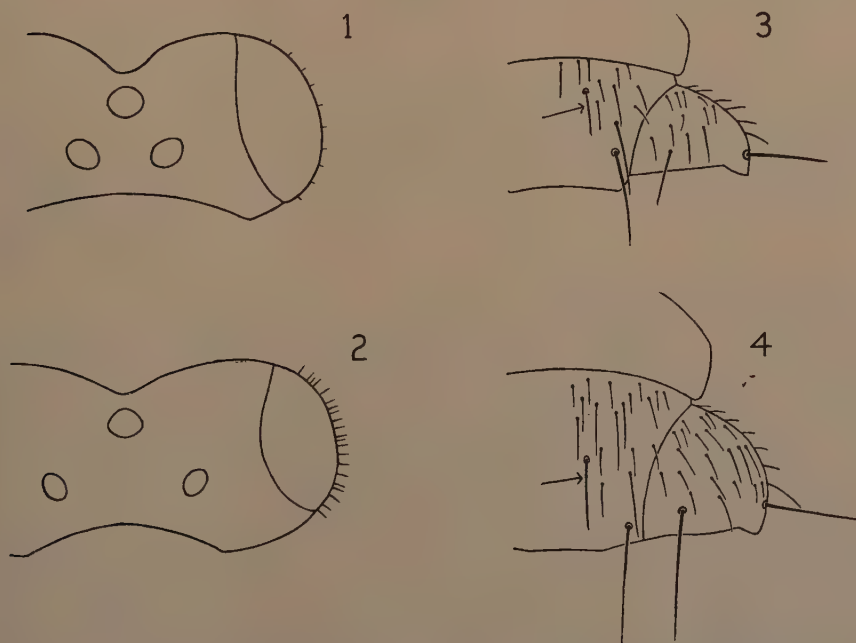
A NEW AUSTRALASIAN SPECIES OF *ELACHERTUS* SPINOLA (HYM.,
CHALCIDOIDEA, EULOPHIDAE) AND NOTES ON TWO
EUROPEAN SPECIES.

By G. J. KERRICH

Commonwealth Institute of Entomology.

So far as known to me, no Australasian species of *Elachertus* has before been described. Girault (1913) did not attribute any Australian species to this genus, nor did he do so in any other of the publications that have been catalogued by Mr. E. F. Riek. It is, however, possible that some species described in other genera may be found to belong here.

A species reared by Mr. R. W. Paine from larvae of *Agonozena* (Lep., AGONOXENIDAE) in Australasia might be run, in Graham's recent key to the British species of *Elachertus* (Graham, 1959), to *charondas* (Wlk.), which differs from it strikingly in having the female petiolar segment strongly transverse, or, more conformably, to the two species in couplet 13-14. These two British and the Australasian species may be separated by the characters given in the following key.



Figs. 1-4.—Dorsal view (left side omitted) of head (figs. 1, 2) and mid lobe of mesoscutum (figs. 3, 4) of *Elachertus agonoxenae* sp.n. (figs. 1, 3) and *E. artaeus* (Wlk.) (figs. 2, 4). Arrow indicates admedian medium-sized hair.

Elachertus Spinola.

Spinola, M., 1811, Ann. Mus. Hist. nat. Paris **17** p. 151. Type species, *Diptolepis lateralis* Spinola 1808.

1. Head (fig. 1) extremely strongly narrowed behind the eyes: ocelli in an acute-angled triangle, POL being slightly less than OOL: eyes relatively weakly hairy, just distinctly so $\times 45$: cheeks very sharply narrowed to mouth: first funicle segment very little longer than second: petiolar segment (female) one and a half times as long as broad: costal cell with an apical half-row of hairs on upper surface and a rather diffuse row on under surface, and bearing very few other hairs: speculum postbasale broader and usually clear: radius shorter, the triangular area between it and the postmarginal almost bare, bearing one to three small hairs: [mesoscutum (fig. 3) as described for *argissa* (Walker)] *agonoxenae* sp.n.

Head (fig. 2) less strongly narrowed behind eyes: ocelli in an obtuse triangle, POL being much greater than OOL: eyes quite strongly hairy, very distinctly so $\times 25$: cheeks less sharply narrowed to mouth: first funicle segment distinctly longer than second: petiolar segment (female) relatively shorter: costal cell more strongly hairy, with two more or less distinct rows on under surface and some other hairs: speculum postbasale narrow, and often bearing several hairs: radius longer, the triangular area between it and the postmarginal about as strongly hairy as the area below it 2

2. Mid lobe of mesoscutum (*cf.* fig. 3) with admedian medium-sized hairs generally arising well forward of half the exposed length of the sclerite, which is also less densely beset with smaller hairs than in alternate; petiolar segment (female) approximately as long as broad: female gaster, less petiole, much longer than broad: a darker species with, in female, the antennal scape and coxae in greater part darkened *argissa* (Walker)

Mid lobe of mesoscutum (fig. 4) with admedian medium-sized hairs arising at about half the exposed length of the sclerite, which is also more densely beset with smaller hairs than in alternate: petiolar segment (female) approximately one and a quarter times as long as broad: female gaster, less petiole, not much longer than broad: a lighter species with, in female, the antennal scape darkened near apex and the coxae, especially the hind ones, with some basal darkening *artaeus* (Walker)

Elachertus agonoxenae sp.n.

Head, thorax and propodeum dark green, or in small specimens steely blue, with pale metallic reflections: gaster at sides and on apical half blackish, with pale reflections; with basal blotch above and most of underside pale testaceous. Antennae having scape pale testaceous, pedicellus pale to rufo-testaceous, and flagellum considerably darkened and with moderate metallic reflections. Legs pale to very pale testaceous, the coxae in greater part darkened but not strongly so: male with coxae more strongly and extensively, and femora slightly, darkened.

Specimens reared in Queensland differ in having the gaster above, in the female, blackish peripherally, but only very little darkened in hinder half discally.

Structure as described in the above key to species and by reference to the key of Graham (1959).

Length 1.2–2.0 mm.

Material.—NEW GUINEA: Lae, 22 ♀♀ (one the holotype) 3 ♂♂, ix.1957, *ex* larvae of *Agonoxena pyrogramma* Meyr. (Lep., AGONOXENIDAE) (R. W. Paine); QUEENSLAND: Tully, 6 ♀♀ 2 ♂♂, ii.1961, *ex* larvae of *Agonoxena* sp. (R. W.

Paine). Holotype ♀ and paratypes in British Museum (Natural History): it is intended that some paratypes shall be sent in exchange to the Australian National Collection, the U.S. National Museum and other institutions. Material of this species has been imported into Fiji for trial against the coconut leaf moth, *Agonoxena argaula* Meyr.

***Elachertus argissa* (Walker).**

1839. *Eulophus argissa* Walker, Monographia Chalciditum 1 p. 172.

Head medium to dark green, thorax, propodeum and gaster dark green to blackish, often with brassy to bronzy reflections, the gaster much less strongly metallic coloured, especially beneath. Antennae rufo-testaceous, usually in greater part darkened and with moderate metallic reflections. Legs rather pale testaceous: coxae in greater part, and sometimes femora slightly, darkened: male with coxae, except at apex, strongly, and femora considerably, darkened.

Structure as described above.

Redescribed from material from the British Isles and France.

Dr. Ch. Ferrière marked as type a female specimen bearing a small rectangular manuscript label "968.", and this specimen I select as lectotype, with the concurrence of Dr. M. de V. Graham. It is a British specimen mentioned under a different manuscript name in the Museum register.

***Elachertus artaeus* (Walker).**

1839. *Eulophus artaeus* Walker, Monographia Chalciditum 1 pp. 172-173.

1878. *Elachistus petiolatus* Thomson, Hymenoptera Scandinaviae 5 pp. 191-193 (synonymy confirmed).

Head, thorax and propodeum medium to dark green, with pale brassy reflections; gaster at sides and in apical half dark green on a blackish background, with basal blotch above and most of underside rufo-testaceous. Antennae rufo-testaceous, considerably darkened and with weak metallic reflections above, but on scape only near apex. Legs rufo-testaceous to paler: coxae, especially the hind ones, usually with some basal darkening.

Structure as described above.

Redescribed from material from the British Isles and Sweden.

I can trace only one Walker specimen in the British Museum collection, a female mounted on a rectangular card, and bearing the label "*artaeus*" in Walker's handwriting. Dr. Ferrière has marked it as type and I hereby validate this, with the concurrence of Dr. Graham. Ferrière also labelled it "*= Elachertus petiolatus* Thoms.", and this confirms the synonymy already suggested by Thomson. The British Museum collection contains a good series of specimens collected in Skåne, Sweden, by D. M. S. & J. F. Perkins in 1938, one of which was used for drawing figs. 2 and 4.

Summary.

A description is given of a new species of *Elachertus*, *E. agonoxenae* (CHALCIDOIDEA, EULOPHIDAE), on the basis of adults reared from larvae of *Agonoxena pyrogramma* Meyr. in New Guinea and *Agonoxena* sp. in Queensland. This species has been imported into Fiji for trial in controlling *A. argaula* Meyr., a pest of coconut palms. The new species is compared with *E. argissa* (Wlk.) and *E. artaeus* (Wlk.), two British species for which lectotypes are here selected.

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OBSERVATIONS ON THE ECOLOGY OF THE COTTON FLEA-BEETLES IN THE SUDAN GEZIRA AND THE EFFECT OF SOWING DATE ON THE LEVEL OF POPULATION IN COTTON.

By E. A. S. LA CROIX *

Entomologist, Ministry of Agriculture, Kenya.

Earlier work in the Sudan Gezira, mainly by M. R. Norman (Joyce, 1955, 1956), has indicated that earlier-sown cotton is more heavily attacked by adult flea-beetles (a mixture of *Podagrica puncticollis* Weise and *P. pallida* (Jacoby), HALTICINAE) than the later-sown cotton, and that within the cotton field the earlier-sown strips are more heavily attacked than the later-sown.

In the Gezira, the land, for the purposes of irrigation, is apportioned into 'numbers', each 90 feddans in area (1 feddan=1.038 acres) and 1,350 metres in length, by 280 metres in width. Each number is divided into nine rectangular tenancies, 10 feddans in area, and 150 by 280 m. in size. One of the shorter sides of the tenancy rests on a watering channel and the other on a road. These sides will henceforth be designated as channel side and road side. Each tenancy is further subdivided into strips or 'angaias', which lie parallel to the channel. In 1954, there were 16 such strips in each tenancy, this number being reduced to 14 in 1955. Sowing is by hand, and at right angles to the greater dimension of the tenancy.

It was suggested by D. G. Pollard (Joyce, 1955, pp. 126-132) that the cotton seedlings become available and attractive to the flea-beetles progressively, owing to the Gezira practice of sowing progressively within the tenancy. The sowing lasts four days, and starts on the channel side of tenancies. If there is a continuous invasion of the cotton by the beetles, a population gradient would be caused.

The experiments described herein were undertaken to test this hypothesis, which could not be proved with the data available because sowing date was always confounded with location in relation to channel and road sides of the tenancy, and also to gain information on the relative infestation of cotton of different initial sowing dates by flea-beetles.

Procedure.

Crop data.

The experiments were carried out on Abd el Galil Block (No. 17) in the Central Gezira, the variety of cotton being X1730A. The sowing dates were as follows:—

	1954	1955
Effective 1st sowing date	16th August	17th August
Effective 2nd sowing date	20th August	19th August
Effective 3rd sowing date	24th August	23rd August
Effective 4th sowing date	27th August	—

Method and layout.

In 1954, three replicates, all of the first sowing date and each consisting of four tenancies, were chosen. Tenancies at the end of the numbers were not used, as it had previously been noted that a second gradient, running from the outer

* Formerly with Fison's Pest Control, Sudan.

long side of the outer tenancy was superimposed upon the existing pattern within the tenancy, and clarity of result was desired. In each replicate, two adjacent tenancies, chosen at random, were sown from the channel side, designated Method I, and the other two from the road side, Method II—in other words, reverse-direction sowing. Random selection on the basis of single tenancy plots would have provided more degrees of freedom for the analysis, but the layout had to be as simple as possible in order that the native foreman in charge of the sowing might not be confused. Each tenancy took four days to sow, four angaias being sown each day.

Examinations were also carried out on randomly chosen replicates in cotton of each of the four sowing dates, in order to investigate the relative degree of infestation.

In 1955, six replicates, each of four tenancies, were used in all, two in each of the first three sowing dates. Each replicate was sown by Method I and Method II as described above. As in this year there were 14 angaias in each tenancy, only two angaias were sown on the final day. Using such a small number of replicates made it possible to examine each tenancy at three-day intervals. The six replicates were all situated within the same small area (although none was near land on which cotton had been grown in the previous year) so that teams could walk to the fields in the event of heavy rain making the road impassable for motor transport.

Sampling.

The basis of the sampling was a count of the adult flea-beetle populations and an estimate of the amount of damage done by them.

Twenty plant holes in each angaia in each sampled tenancy were examined by the teams. Each team consisted of five native assistants spaced at intervals of two rows, forming an inclined line. The team moved along one longer edge of the tenancy, the outside assistant counting in the outside row of the crop. Without a change in their positions relative to each other, counting was continued on the other side of the tenancy. Two evenly spaced belts in the middle of the tenancy were also counted in this way.

The sampling of the flea-beetle population was undertaken by means of a

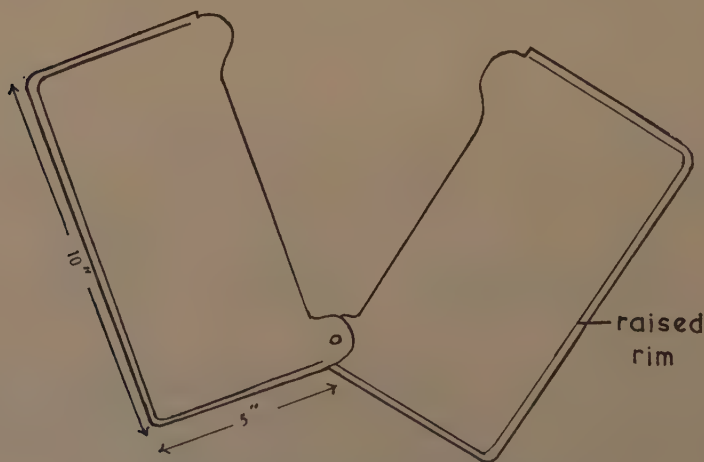


Fig. 1.—Metal tray for sampling flea-beetle populations.

two-piece, white-painted metal tray, hinged at one side, which could be placed around the base of the seedlings, and completely closed, so as to present a white area completely surrounding the plant hole. When the tray had been gently placed in position, the seedlings were patted from above. This caused the beetles to jump off the plant on to the tray, where they could easily be counted on the white background. The number of beetles counted at each plant hole was recorded.

The results of sampling beetle populations were found to be very variable, and no great reliance could be placed on them, except to determine the time at which the peak population occurred. This great variability may be due in part to a sampling error, but it is considered that the habits of the beetles play a large part in it. In the early morning, moderate numbers were observed on the plants, during the middle of the day, few beetles were seen, but in the evening large numbers appeared, and extensive mating occurred. As replicates were of necessity sampled at different times in the day, the habits of the beetles were reflected in the counts obtained.

The flea-beetles damage the cotton plant by feeding on the cotyledons and leaf. They eat a small rounded patch of material and move on to continue feeding on an intact area. A damaged leaf or cotyledon therefore presents a perforated appearance, the holes tending to join up about 18 days after sowing, which is about the time that the plant ceases to be so dependent on its cotyledons. At the time of year in which the investigations were undertaken, no other insect causes damage of the same nature as that of the flea-beetles. A caterpillar, *Amsacta*

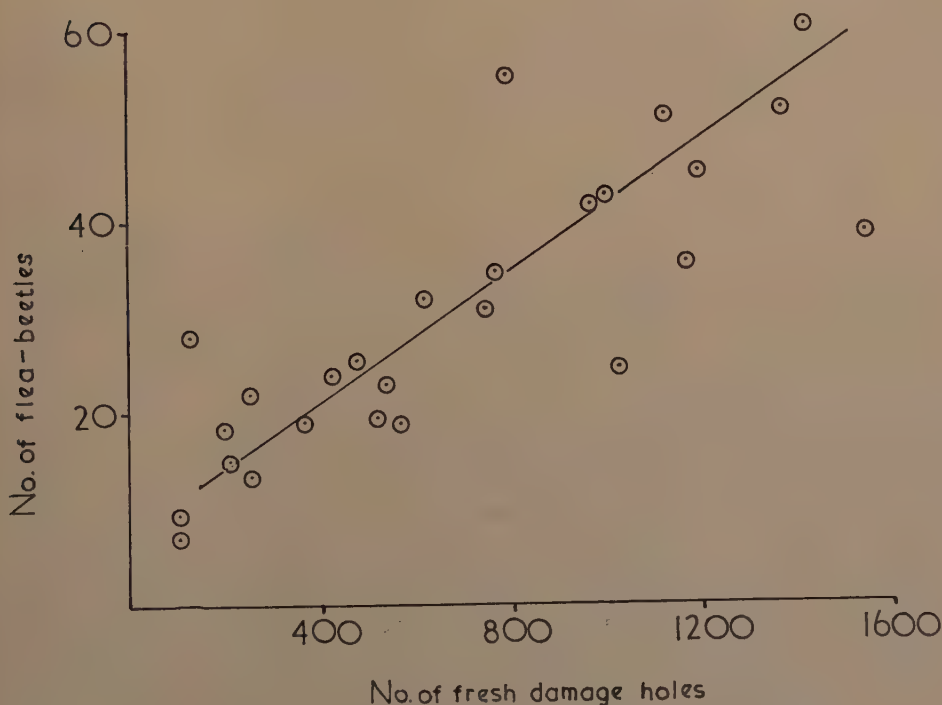


Fig. 2.—Relationship between average total numbers of flea-beetles and average total associated numbers of fresh damage holes per tenancy.

TABLE I.

Distribution of flea-beetle damage across tenancies, 1954. Number of fresh holes* per replicate per angaia.

Date	Days after sowing	Angaia																Level of significance between angaia 2 and 16		
		Angaia																Total		
		2	4	6	8	10	12	14	16	Total	2	4	6	8	10	12	14			16
29 Aug.	..	160	125	157	117	109	93	54	72	887	91	64	77	82	61	78	76	137	666	5%
8 Sept.	..	11	32	0	4	11	6	44	43	151	49	33	18	20	42	34	51	0	247	N.S.
	Total	171	157	157	121	120	99	98	115	1038	140	97	95	102	103	112	127	137	913	5%

* Here, and in subsequent tables, these are feeding holes of flea-beetles in the cotyledons and leaves. The number of fresh holes is obtained by subtracting the previous total from the current total, a negative result is recorded as zero.

N.S. signifies not significant.

moloneyi (Druce), causes the nearest approximation to flea-beetle damage, but the holes are irregular, not rounded or regular as are flea-beetle holes, and patches of the lower epidermis are left, which again is not the case with the flea-beetles. The assistants were easily trained to distinguish between damage caused by flea-beetles and that by *Amsacta*.

When the number of flea-beetles in each plant hole had been counted, the number of complete holes in the cotyledons and leaves caused by the beetles was recorded. This method of assessing flea-beetle damage is rather arbitrary, in that it takes no account of the size of the hole, but preliminary observations had indicated that it would be sufficient for the type of experiment undertaken, and it has also, in its essentials, been used by Alexander & others (1944). In the present work, the amount of fresh flea-beetle damage is assumed to be proportional to the numbers of beetles present. As this method of assessment of population is very open to criticism, its validity was examined by data from the experiment itself. The flea-beetle counts were very variable, and so the scale of comparison, as it were, had to be large enough to absorb undue variations, but small enough to provide a sufficiency of actual points. The scale chosen was the average per tenancy per replicate. A graph of the points plotted in comparing average total flea-beetle numbers with the average total associated number of fresh holes per tenancy is presented in fig. 2. Data from each year and from cotton not more than 18 days old are used, but examples in which very low flea-beetle counts were obtained (*i.e.*, those taken about noon) have been excluded. The graph seems to be curvilinear but, for the portion in which most of the records occur, a simple linear correlation would appear to apply. On analysis, a correlation of 0.66 was obtained. This is very highly significant by Student's *t* test.

Except when otherwise indicated, the figures quoted below were obtained from unthinned cotton. The figures from thinned cotton may be comparable, but the diminution in the number of plants and therefore the amount of damage in the plant hole, causes the author to treat them with reservations. Thinning involves reducing the number of plants per hole to three in the case of the first and second sowings and to five in the case of the last two sowings. It is usually done about the middle of September.

Results.

Gradient within the tenancy.

As mentioned before, in 1954 the three replicates in this part of the experiment were all of the first sowing date. The damage occurring across the tenancies, from the channel to the road side, is recorded in Table I. In order to obtain a more even representation, the means of each group of two angaias are used. These angaias are numbered from the channel side. The results show that a significant gradient of damage exists across the cotton field on the 13th day after sowing, the earlier-sown cotton being more heavily attacked, but that fresh damage after this and by the 8th September (23rd day after sowing) does not conform to this pattern, although the total damage done over the counting period retains its bias.

In determining whether or not this distribution was due to chance, a random block analysis was carried out on the differences between the amount of damage on the channel side and that on the road side. This difference was positive in the case of Method I and negative in Method II.

In 1955, a split block design was adopted, the normal and reverse-direction techniques being applied to the first three sowings. The results are shown in Table II. The data have been presented by sowings.

The over-all tendency seems to be for cotton to be attacked in accordance with the sowing differential up to about 14-15 days after sowing and for the attack to

TABLE II.
Distribution of flea-beetle damage across tenancies, 1955. Number of fresh holes per replicate per angaia.
Direction of sowing \rightarrow \leftarrow

Sowing	Date	Days after sowing	Angaias														Level of significance between angaias 2 and 14											
			2				4				6				8				10				12				14	
First	28 Aug.	11 (± 1)	134	115	99	98	76	78	61	661	53	59	76	68	84	110	130	580	5%									
	31 Aug.	14 (± 1)	74	101	92	90	90	58	86	591	53	71	88	97	103	112	102	626	5%									
	4 Sept.	18 (± 1)	90	65	77	79	100	117	162	690	95	60	26	20	6	0	0	207	5%*									
Second	Total		298	281	268	267	266	253	309	1942	201	190	190	185	193	222	232	1413	N.S.									
	28 Aug.	9 (± 1)	33	27	26	26	13	20	24	169	29	21	21	24	25	28	40	188	N.S.									
	31 Aug.	12 (± 1)	21	34	10	0	7	5	0	77	0	0	7	0	0	21	4	32	5%									
Third	4 Sept.	16 (± 1)	75	12	46	45	57	49	78	362	81	64	79	84	64	52	61	485	N.S.									
	Total		129	73	82	71	77	74	102	608	110	85	107	108	89	101	105	705	N.S.									
	31 Aug.	8 (± 1)	31	37	30	26	31	24	18	197	22	21	27	20	24	25	33	172	5%									
	4 Sept.	12 (± 1)	8	4	20	21	16	26	33	128	28	35	28	23	29	22	22	187	N.S.									
	Total		39	41	50	47	47	50	51	325	50	56	55	43	53	47	55	359	N.S.									

* Against sowing differential.

TABLE III.

Distribution of flea-beetle damage across tenancies. Number of fresh holes per angaiia.

Date		No. of days after sowing	Angaiias															
			Direction of sowing←→															
			2	4	6	8	10	12	14	Total	2	4	6	8	10	12	14	Total
27 Aug.	..	11	17	20	17	32	44	49	35	214	36	63	13	15	6	13	10	156
31 Aug.	..	15	13	10	38	9	4	81	68	223	71	58	62	24	36	34	34	319
4 Sept.	..	19	142	91	88	179	228	145	108	981	137	128	96	206	204	146	306	1223

be *against* the gradient after this, thereby evening out the damage throughout the tenancy.

Also, in 1955, an unreplicated observation was made on the behaviour of the flea-beetles when a large area of cotton was sown from the centre. To do this, two adjacent numbers were used, being sown from the road and channel which divided them. The same type of assessment was carried out on these areas as in the rest of the experiment.

No statistical analysis was carried out, but the results (Table III) are obviously in complete agreement with those made on the main body of the experiment.

Differences in level between sowings.

The figures for 1954 only allowed comparisons to be made of total damage between cotton of different sowing dates on two occasions, the 10th September (Table IV) and on the 30th day after sowing (Table V) for cotton of each sowing date.

TABLE IV.

Sowing-date means of flea-beetle damage holes (average no. per replicate) for 10th (± 2) September 1954.

	1st sowing I	2nd sowing II	3rd sowing III	4th sowing IV
Days after sowing	25 (± 2)	21 (± 2)	17 (± 2)	14 (± 2)
No. of holes ..	6292	4294	3322	546

L.S.D. for comparing I with II, III & IV = ± 792 .

L.S.D. for comparing II with III = ± 1103 .

L.S.D. for comparing IV with II & III = ± 1634 .

(L.S.D. = Least significant difference.)

TABLE V.

Sowing date means of holes (average number per replicate) for 30(± 2) days after sowing, 1954.

1st sowing I	2nd sowing II	3rd sowing III	4th sowing IV
5424	4906	4916	1550

I, II & III did not differ significantly. L.S.D. for comparing IV with I, II & III = ± 1391 .

All sowings had been thinned by the time of the above examination.

The deductions from Tables IV and V would seem to be that the earlier-sown cotton initially suffered more damage, but that later on there was no statistical difference between the levels of damage in the first three sowings. The over-all picture, however, is obviously that the level of damage decreases with the order of lateness of sowing.

In 1955 (see Table VI), the counts were more exactly comparable on a calendar date basis.

The rate of increase in damage was approximately the same on cotton of equal age, although the exception of the second-sown cotton on the 12th day must be noted. The earlier the sowing, however, the greater is the total damage that has been done by 4th September. After this date, thinning obscures the results.

TABLE VI.

Sowing-date means of fresh holes (average number per replicate) for August and September 1955.

Date of examination	28 Aug.	31 Aug.	4 Sept.	Total
1st sowing				
No. of days after sowing ..	10	14	18	
No. of fresh holes ..	2492	2378	1768	6638
2nd sowing				
No. of days after sowing ..	8	12	16	
No. of fresh holes ..	674	180	1752	2606
3rd sowing				
No. of days after sowing ..	—	4	8	
No. of fresh holes ..	—	734	630	1364
Level of significance ..	N.S.	5%	5%	5%
L.S.D.	—	± 981	± 732	± 2996

Time of peak population.

As mentioned before, great variation occurred in the adult flea-beetle figures. The over-all results are clarified by taking the mean of the figures for a particular date, irrespective of sowing date (Table VII).

TABLE VII.

Average number of flea-beetles per replicate, irrespective of sowing date.

1954	—	29 (±1) Aug.	4 (±1) Sept.	8 (±1) Sept.	12 (±1) Sept.
		155	546	292	126
1955	28 (±1) Aug.	31 (±1) Aug.	4 (±1) Sept.	7 (±1) Sept.	12 (±1) Sept.
	111	260	183	122	81

To generalise from Table VII, flea-beetle populations on cotton are heaviest at the very end of August and during the first week of September.

Discussion.

The use of flea-beetle damage as a basis for findings would seem to be satisfactory in an investigation of this kind, because the period during which damage holes remain separate, and therefore produce usable data, coincides with the period over which the beetle attacks are of most economic importance. The effect of cotton flea-beetle attacks at different stages of the plant's growth is not precisely known, but to judge from information available on other Halticine beetles and on past experience in the Gezira, the earlier damage is the most deleterious. Once the plant has produced leaves it can grow away from the damage.

The foregoing results indicate that there is a gradient of damage coinciding with the sowing gradient on the first-sown cotton up to the 14th day after sowing of the cotton. Thereafter the gradient levels out and develops against the sowing gradient after 19 days (see Tables I, II, III). This infers that the flea-beetles within the tenancy leave cotton sown about 19 days previously and search actively for younger cotton. The severity of the attack would seem to diminish with the lateness of sowing (see Tables II, IV, VI) but tends to even out when the plants have been exposed to attack for the same length of time (see Tables V and VI).

To expand on Table IV, wherein the sowing date means for the 10th September are given, assuming that the seeds take four days to germinate, cotton of the four sowings had been exposed for, respectively, 21, 17, 13 and 10 days. This gives figures for holes per day since emergence for the successive sowings of 299, 253, 255 and 55. By the 30th day after sowing, see Table V (and after thinning, which probably affected counts on all equally) the difference between the first three sowings had largely disappeared, the corresponding figures then being 209, 189, 189 and 60. Clearly the fourth sowing suffered a less severe attack than the other sowings.

It seems reasonable to deduce from Table VII that the peak of flea-beetle population occurs in the period about 31st August to 4th September. At this time, in normal Gezira practice, the first-sown cotton is about 14–18 days old (from date of sowing), the second sowing 11–15 days, the third 7–11 days and the fourth about 4–8 days old. As most of this peak obviously migrates on to the first-sown cotton, for this is clearly the most severely attacked, it would seem that cotton at this stage is most attractive to the beetles. After this peak, the last three sowings would in turn become attractive to the beetles, but as the population is diminishing, the attacks lose their severity. The fourth-sown cotton would only become attractive about 10th September when the population level is well below what it was 6–10 days earlier.

These findings have obvious practical implications for control measures, when necessary. Drift spraying is usually employed in the Gezira to control serious outbreaks of this pest as it is usually impossible to put a sprayer through the cotton fields at this time of year, and the drift sprayer exploits the edge effect of beetle distribution. To be most effective, the drift spray should be applied from the earlier-sown side of the cotton field, before the cotton is more than 18 days old. As a gradient in accordance with the sowing differential was obtained in the cotton sown by Method II, the use of this sowing technique would be of value where it would follow the direction of the prevalent wind at the time of sowing (S.W.) and would thereby enable a drift sprayer to be used effectively. The order of priority obviously decreases with the lateness of sowing. Spraying after the first week of September would probably be unnecessary, owing to the decline in flea-beetle numbers by that time, although a population that was extremely high at its peak might, although in decline, still be great enough to damage the fourth sowing.

Summary.

A series of experiments was undertaken to examine the relative concentration of cotton flea-beetles (*Podagrica puncticollis* Weise and *P. pallida* (Jacoby), HALTICINAE) within cotton fields in the Sudan Gezira in 1954 and 1955. Assessments were carried out on the flea-beetle populations and on the damage done by them, and evidence is produced to show that the use of a damage assessment in the first three weeks of the life of the cotton plant is more satisfactory in a large-scale experiment than beetle counts. The assessment lasted for between three and four weeks after the emergence of the seedling cotton.

It appeared that the beetles tended to leave cotton 19 days after sowing. Damage within the cotton field was at first in accord with the sowing differential,

but then shifted against this as the flea-beetles moved on to the later-sown cotton as this became more attractive.

The peak population of flea-beetles occurred during the last few days of August and the first few of September when the first-sown cotton was 14–18 days old, and this suffered more damage than the later-sown and less attractive cotton. Differences in the level of attack on the four sowings tended to even out, but did not do so completely as the flea-beetle population was declining.

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OBSERVATIONS ON *CARCELIA EVOLANS* (WIED.) (DIPTERA,
TACHINIDAE), A PARASITE OF *DIPAROPSIS WATERSI* (ROTHS.)
(LEPIDOPTERA, NOCTUIDAE), IN NORTHERN NIGERIA.

By W. REED and M. A. CHOYCE

Empire Cotton Growing Corporation, Ministry of Agriculture Research
Station, Samaru, Zaria, Northern Nigeria.

(PLATE IX.)

In Northern Nigeria, the red bollworm, *Diparopsis watersi* (Roths.), is parasitised by a Tachinid fly at first regarded as close to *Carcelia illota* (Curr.) but later thought to represent a form of *C. evolans* (Wied.) (Pearson, 1958). The type locality of the species is Sierra Leone, and, as at present understood, *C. evolans* is widely distributed and polyphagous. It is a common parasite of *D. watersi* in the neighbouring territory of Tchad (Galichet, 1957) and has been recorded from several Lepidopterous hosts in Africa south of the Sahara, as well as from a number of countries in the Far East. There is as yet insufficient evidence to define the exact status of the Nigerian form within the larger complex.

Biology.

Egg and larval stages.

There is no recorded information about the egg or early larval stages. We have not observed oviposition and when newly emerged male and female flies were caged in the laboratory with various instars of *D. watersi* and provided with water, sugar, and cotton plants, no pairing was seen and all the flies died within ten days. Subsequent examination of females revealed no eggs. Late-instar larvae of the parasite quickly died when removed from their host larvae, and attempts to rear them on macerated larvae did not succeed.

The *Diparopsis* pupae found to contain *Carcelia* and used in the investigations described in this paper were obtained by collecting cotton bolls containing fifth-instar larvae and placing them in troughs containing soil. The fully grown larvae leave the bolls and enter the soil before pupating. Although the troughs were unscreened, they were some distance from the nearest cotton field and it is therefore inferred that parasitism occurs before the fully grown bollworm leaves the cotton plant to enter the soil.*

Pupation and emergence of the parasite.

Before pupating, the fully grown larva of *Diparopsis* burrows below the surface of the soil and surrounds itself with an earthen cell. *C. evolans* is a larval parasite that normally pupates outside its host but within the cell the host has formed. The typical position of the host and the puparium of the parasite is shown in Plate IX, fig. 1. In the laboratory, parasite larvae have only been seen to leave their host immediately before, or after, the host has pupated. As puparia of the parasite have never been found in cotton bolls, it is unlikely that, under field conditions, the parasite leaves its host before the latter enters the soil to pupate.

The wall of the cell formed by *Diparopsis* consists of soil particles closely

* More recent observations on pupae obtained from screened troughs support this conclusion.

cemented together and is lined with silk. The considerable force required to break open the cell is applied by the moth, when ready to emerge, through its heavily chitinised frontal process. Observations on cells containing parasitised pupae of *Diparopsis* show that *C. evolans* has overcome the difficulties involved in escaping from the cell by the following method: after leaving its host, and before forming its puparium, the fully grown parasite larva excavates a small pit, 2-3 mm. in diameter, through the silk layer and wall at one end of the cell (Pl. IX, fig. 2). In the laboratory, this takes place in dry cells; under natural conditions in the soil, the cell-wall would be moist (Choyce & Reed, 1961) and thus easier to penetrate. The puparium is then formed with its anterior end lying close to the pit. At this stage, a *Diparopsis* cell containing a parasite can often, but not always, be distinguished externally by the presence of a pin-sized hole at one end, where the exit pit has broken through to the outside (Pl. IX, fig. 3, extreme right). When the fly is ready to emerge, vigorous expansion and contraction of the ptilinum splits and forces off the cap of the puparium. It is presumed that the fly then uses its ptilinum to push a way through the weakened cell-wall at the exit pit. In the field, after emerging from a cell buried in the soil, the fly must force its way to the surface. On those occasions when a fly has been observed emerging from soil in pupation troughs, the ptilinum was still expanding and contracting vigorously.

Parasite mortality.

The proportion of parasites which successfully emerge in the laboratory is very variable and appears to be easily affected by the conditions under which the cells of *Diparopsis* are stored. During 1960, 78 parasites made normal emergences from 86 parasitised long-term pupal cells set singly in tubes plugged with cotton-wool. In the same year, however, only 123 flies emerged normally from some 250 cells that were found on examination to have contained parasites and that had been stored in tins containing a large number of other cells. Of the flies that should have emerged from the remainder, 51 died whilst leaving the cell and 68 dried up within the puparium.

The newly emerged fly exhibits a marked tendency to push through small holes. This obviously assists escape from the cell and the soil, but in certain circumstances it can cause the fly's death. Several examples have been found in which the fly has failed to find its exit pit, but instead has entered the pupal skin of its host through the hole from which it emerged as a larva, there jamming itself in the posterior end. Similarly, some flies had re-entered their puparia head first, and were unable to leave.

Short- and long-term generations.

The life-cycle of *C. evolans* is closely adapted to that of *D. watersi*; in both insects, short-term generations during the cotton-growing season alternate with a long-term or diapause generation, which survives the dry season.

During the cotton season, from June until November, the pupal period of *D. watersi* in the laboratory varies between 11 and 31 days (Reed & Choyce, unpublished data). The pupal period of *C. evolans* is more constant, with a range of 12-14 days under similar temperature conditions ($27 \pm 5^\circ\text{C}.$). As November advances, an increasing proportion of larvae of *D. watersi* form long-term pupae (Geering & Baillie, 1954). Evidence suggesting that the incidence of diapause in *C. evolans* is rather later than in its host is discussed below (p. 789).

The stage in its development at which *C. evolans* goes into diapause, and the factor or factors inducing diapause, are unknown. Between December 1959 and March 1960 over 200 individuals from a collection of diapause pupae of *D. watersi* were carefully dissected, but no trace of the parasite could be found. Further samples, totalling 1,262 cells, of the same collection were subsequently shown to contain 303 parasites. It seems probable, therefore, that *C. evolans* goes into

diapause as a very small first-stage larva, which cannot be easily distinguished from the contents of the host pupa.

The maximum larval life is given as 90 days in Tchad (Galichet, 1957), but in Nigeria flies have emerged more than a year after pupation of the host. The mean duration of diapause in dry conditions, inside and outside the laboratory, and in pupation troughs, is shown in Table I. The pupal cells stored outside the laboratory were subjected to a greater diurnal variation in temperature than those inside, and, unlike those in the pupation troughs, were sheltered from rain.

TABLE I.

Duration of diapause in *D. watersi* and *C. evolans*. Data from collections of fifth-instar larvae of *D. watersi* made on cotton at Daudawa and Shemi, 1959. The numbers of individuals on which observations were made are shown in brackets.

Place of storage	Dates of collection	Mean length of diapause* (weeks)	
		<i>D. watersi</i>	<i>C. evolans</i>
Inside laboratory	13—28.xi	26.5 (165)	30.3 (27)
	29.xi—24.xii	22.9 (138)	27.3 (33)
Outside laboratory	13—28.xi	36.2 (64)	38.8 (37)
	29.xi—24.xii	31.0 (88)	35.8 (26)
In pupation troughs	13.xi—24.xii	42.2 (312)	36.1 (23)

* Reckoned as the period from collection of fifth-instar larvae to emergence of adult moth or fly, and therefore including the period of morphogenesis.

Both inside and outside the laboratory, the mean duration of diapause was greater in the parasite than in the host. In pupation troughs, on the other hand, with conditions approximating to those in the field, the duration of diapause was greater in the host than in the parasite.

The mean pupal period of *D. watersi* was longer in pupation troughs than in the laboratory; this confirms earlier observations (Choyce, 1955). The effect of the external environment seems to be less marked in the case of *C. evolans*, for the length of diapause of parasites from pupation troughs was similar to that of parasites from pupae stored outside the laboratory. This is further shown in Table II, which gives a summary of the monthly emergence of moths and parasites from pupae in pupation troughs during 1959 and 1960. The spreadover of parasite emergence was more regular than that of moths, and there was no peak emergence of parasites in October. However, this conclusion requires further confirmation, as it is possible that some flies escaped from the troughs, and were not included in the counts (see below).

TABLE II.

Monthly emergence of *D. watersi* and *C. evolans* from pupation troughs. Monthly emergences from long-term pupae, expressed as a percentage of total emergence, obtained in 1959 and 1960.

	May	June	July	August	September	October	November
<i>D. watersi</i>	3	9	5	10	18	53	2
<i>C. evolans</i>	0	27	15	23	27	8	0

TABLE III.

Short- and long-term emergence of *D. watersi* and *C. evolans* from collections of fifth-instar larvae of *D. watersi* made in 1959.

Origin of material	Dates of collection of larvae	No. of pupal cells set ¹	Short term ²		Pupal cells remaining after short-term emergence ³	No. of cells used for long-term emergence ³	Long term ⁴		Remainder ⁵	
			<i>D. watersi</i>	<i>C. evolans</i>			<i>D. watersi</i>	<i>C. evolans</i>	Live pupae	Dead pupae
Sprayed cotton, Daudawa	13-28.xi	1002	16	27	959	318	229	44	21	24
	29.xi-24.xii	674	4	20	650	273	157	87	5	24
Unsprayed cotton, Sheni	13-28.xi	1410	7	137	1266	279	158	87	16	18
	29.xi-24.xii	1433	2	46	1435	392	262	85	24	21

¹ When pupal cells from which neither moths nor parasites had emerged were examined in December 1960, it was found that some 3-5 per cent. of the larvae had not formed pupae. These have been excluded from the totals given here.

² Short-term emergences are regarded as those occurring within five weeks of collection of the larvae. They include fully developed moths or flies that had failed to emerge and that were found when cells suspected of having dead contents were examined after five weeks.

³ Samples were taken from pupal cells remaining after short-term emergence of having dead contents were examined after five weeks.

⁴ These numbers include, in addition to observed emergences, those moths and flies that were found when the pupal cells were dissected in December 1960, and that had died while attempting to emerge or were recognisable as adults within the pupal cell.

⁵ Determined by dissection of pupal cells from which no emergence had occurred, in December 1960.

Incidence of *C. evolans* on *D. watersi*.

Pupation-trough records at Samaru prior to 1959 showed that the over-all rate of parasitism of *D. watersi* by *C. evolans* (expressed as a percentage of the original number of viable pupae set) was less than 10 per cent. The actual incidence almost certainly exceeded this figure, since it has now been demonstrated that the fly can escape from the cages used to obtain those records. The tendency for newly emerged flies to push through very small holes has already been noted. In the laboratory, parasites have been observed squeezing through holes, of less than 0.1 in. diameter, in perforated zinc similar to that used for the cages. It is therefore reasonable to assume that escapes have occurred from these cages in the past. Confirmation of this has been obtained by comparing pupation-trough and laboratory emergences. From 800 long-term pupae set in troughs, 82 parasites were obtained, equivalent to 10.3 per cent. Laboratory samples from the same collection yielded 303 parasites from 1,262 pupal cells, a parasitism rate of 24.0 per cent.

A more precise estimate of the incidence of the parasite was made in 1959-60 at Daudawa and Shemi, where the early stages of the southern Katsina cotton-seed multiplication scheme are grown (Leonard, 1954). In 1959, 230 acres of cotton, which had been sown between 22nd June and 6th July on the Main Farm at Daudawa, were treated three times during the period 18th September to 4th November with a spray mixture containing DDT and γ BHC, at 1.3-1.8 and 0.2-0.3 lb. per acre, respectively, applied by tractor-mounted sprayer at 12 gal. per acre. These sprays were primarily intended to control stainers, *Dysdercus supersticiosus* (F.), and Mirids, *Campylomma* spp.; it was not expected that effective control of *D. watersi* would be attained with the concentration of insecticides used.

Collections of bolls containing fifth-instar larvae of *D. watersi* were made from 13th November until 24th December, and placed in troughs containing dry soil, where the larvae were allowed to pupate, the pupal cells being obtained by sieving the soil two or three days later. Similar collections were made on 150 acres of unsprayed multiplication cotton grown at Shemi, some four miles from Daudawa.

An analysis of the short- and long-term emergences of moths and parasites from these collections is given in Table III. In this table, short-term emergences of both moths and parasites have been regarded as those which occurred not later than five weeks from the collection of the fifth-instar larvae. In addition to living moths and parasites, those which died during emergence, or were recognisable as moths or parasites within the pupal cell when this was dissected, have been included in the totals of long-term emergences.

The figures given in Table III have been used to calculate the proportion of short- and long-term moths and parasites, expressed as a percentage of the original number of pupal cells set (Table IV). From Table IV it will be seen that 23.1 per cent. of pupal cells derived from sprayed cotton at Daudawa were parasitised, compared with 30.7 per cent. from unsprayed cotton at Shemi. The comparatively low rate of parasitism (16.0 per cent.) for collections of larvae made between 13th and 28th November at Daudawa may have been due to the application of insecticide to the cotton earlier in the season.

The proportion of hosts and parasites entering diapause from larvae collected between 13th November and 24th December 1959, is shown in Table V; calculations have been based on the number of long- and short-term emergences given in Table III. Taking the material as a whole, 99 per cent. of the moths, and 82 per cent. of the parasites were long term. There was little difference between the earlier and the later collections as regards the proportions of moths that were long term, but the former yielded a substantially smaller proportion of long-term parasites than did the latter, suggesting that the onset of diapause in the parasite is later than it is in its host.

TABLE IV.

Short- and long-term emergence of *D. watersi* and *C. evolvans* from collections of fifth-instar larvae of *D. watersi* made in 1959, expressed as a percentage of the number of pupal cells set (calculated from Table III).

Origin of material	Dates of collection of larvae	No. of pupal cells set ¹ (=100%)	Short term ³		Long term ⁴		Remainder ⁵		Total emergence (to 15 Dec., 1960)	
			<i>D. watersi</i>	<i>C. evolvans</i>	<i>D. watersi</i>	<i>C. evolvans</i>	Live pupae	Dead pupae	<i>D. watersi</i>	<i>C. evolvans</i>
Sprayed cotton, Daudawa	13-28.xi	1002	1.6	2.7	69.0	13.3	6.3	7.2	70.6	16.0
	29.xi-24.xii	674	0.6	3.0	55.5	30.7	1.8	8.5	56.1	33.7
All Daudawa collection		1676	1.2	2.8	63.5	20.3	4.5	7.7	64.7	23.1
Unsprayed cotton, Shemi	13-28.xi	1410	0.5	9.7	50.8	28.0	5.2	5.8	51.3	37.7
	29.xi-24.xii	1483	0.1	3.1	64.7	21.0	5.9	5.2	64.8	24.1
All Shemi collection		2893	0.3	6.3	57.9	24.4	5.6	5.5	58.2	30.7
Total collection		4569	0.6	5.0	60.0	22.9	5.2	6.3	60.6	27.9

¹, ², ⁴, ⁵ See footnotes to Table III.

TABLE V.

Proportion of individuals of *D. watersi* and *C. evolans* entering diapause, expressed as a percentage of total short- and long-term emergences (calculated from Table III).

Origin of material	Dates of collection of larvae (1959)	Calculated total short- and long-term emergences ¹		% Short term		% Long term	
		<i>D. watersi</i>	<i>C. evolans</i>	<i>D. watersi</i>	<i>C. evolans</i>	<i>D. watersi</i>	<i>C. evolans</i>
Sprayed cotton, Daudawa	13-28.xi	707	160	2.3	16.9	97.7	83.1
	29.xi-24.xii	378	227	1.1	8.8	98.9	91.2
Unsprayed cotton, Shemi	13-28.xi	723	532	1.0	25.8	99.0	74.2
	29.xi-24.xii	961	357	0.2	12.9	99.8	87.1
Total	13.xi-24.xii	2769	1276	1.0	18.0	99.0	82.0

¹ Estimated from actual number of short-term emergences *plus* number of long-term emergences calculated from samples observed.

It is evident from this result that short-term parasites do not necessarily always emerge from potentially short-term pupae of *D. watersi*. Neither can it be assumed that long-term parasites only emerge from long-term *D. watersi* pupae. These conclusions are unexpected. If *C. evolans* parasitises larvae of *D. watersi* at random, and if diapause in the parasite is determined by some 'factor' in the tissues of the host, then the proportions of host and parasite which enter diapause should be the same. It is difficult to believe that the parasite could show a preference for potentially short-term bollworms. Although there is no evidence on this point, it is perhaps reasonable to suggest that the young stages of *C. evolans* are influenced by some factor differing in nature, or in intensity, from that which induces diapause in *D. watersi*.

Discussion.

There is no doubt that *C. evolans* can exercise a considerable degree of control of *D. watersi* in Northern Nigeria, and its importance is greater than earlier reports on its incidence would indicate (Choyce & Emsley, 1956). The incidence of parasitism amongst the long-term fraction of the population, amounting to over one quarter in the combined samples obtained from Daudawa and Shemi during 1959 (Table IV), is particularly significant, since a large proportion of the population of moths is univoltine. A similar high rate of parasitism, rising to 25 per cent. in December, is reached in Tchad Republic (Galichet, 1957).

Because of the value of *C. evolans* as a factor in the control of *D. watersi*, the effect of insecticides on its incidence is obviously a matter of some concern. At present, the use of insecticides on cotton in Nigeria has not extended far beyond the experimental stage, except at Samaru, and on the early stages of cotton multiplication in southern Katsina. Results have, however, been encouraging, and it is likely that cotton insecticides will gradually be more widely used. On theoretical grounds it is to be expected that the parasite will be much easier to kill with insecticides than the bollworm, which is notoriously difficult to control. That this is probably occurring in practice is suggested by the results obtained in 1959 (Table IV). Here the incidence of parasitism during November at Daudawa, a few weeks after completion of insecticide applications there, was less than half the incidence recorded there later in the season, or concurrently on unsprayed cotton at Shemi. The majority of long-term pupae are formed in November, following the peak emergence of moths in early October. The effect on the carryover of moths to the following season of any reduction in the incidence of the parasite at this time is likely to be substantial. It is hoped that further observations on this subject, and on the biology of *C. evolans*, will enable spraying schedules to be devised that will have a minimum effect on the beneficial activities of the parasite.

Summary.

Carcelia evolans (Wied.) is a common parasite of *Diparopsis watersi* (Roths.) in Northern Nigeria. The egg and early larval stages have not been observed, but the latter are thought to be passed in the larva of the host. The fully grown parasite larva appears to leave its host after the latter has entered the soil and formed its pupal cell, and either immediately before, or after, the host pupates. The parasite puparium is thus formed inside the pupal cell of *D. watersi*, but outside the host. Before forming its puparium, the parasite larva excavates a pit in the wall of the cell, thus facilitating the subsequent exit of the adult fly, which can push through the weakened cell at this point.

The life-cycle of *C. evolans* is closely adapted to that of its host, with short-term generations during the cotton season alternating with a long-term, or diapause, generation during the dry season. It is believed that *C. evolans* goes into diapause as a minute first-stage larva, but the factors that induce diapause are unknown.

The short-term pupal period of *C. evolans* (12–14 days) was less variable than that of *D. watersi* (11–31 days) at $27 \pm 5^\circ\text{C}$. In dry conditions, inside and outside the laboratory, the mean duration of diapause for *C. evolans* (27.3–38.8 weeks) was longer than that of *D. watersi* (22.9–36.2 weeks). In pupation troughs, approximating to field conditions, the mean duration of diapause in *D. watersi* (43.2 weeks) was greater than that of its parasite (36.1 weeks). The spreadover of emergence of the parasite from pupation troughs was more regular than that of moths, with no peak in October.

Earlier estimates of the incidence of parasitism at Samaru are probably inaccurate because adult flies have now been shown to be capable of escaping through the apertures of the perforated zinc of the cages then in use. Estimates of the rate of parasitism, made in southern Katsina, showed that 23.1 per cent. of pupal cells obtained at Daudawa between 13th November and 24th December 1959 from cotton that had earlier been treated with insecticidal sprays were parasitised, compared with 30.7 per cent. of those obtained in the same period from unsprayed cotton four miles away. The rate of parasitism was particularly low (16 per cent.) amongst the larvae collected at Daudawa in November, due possibly to the insecticide applications that had been made earlier in the season.

These results imply that the parasite is easier to kill than its host, and an increasing use of insecticides on cotton in Northern Nigeria may therefore adversely affect the degree of control achieved by the parasite.

Acknowledgements.

We are indebted to the Minister of Agriculture, Northern Nigeria, for permission to publish this paper.

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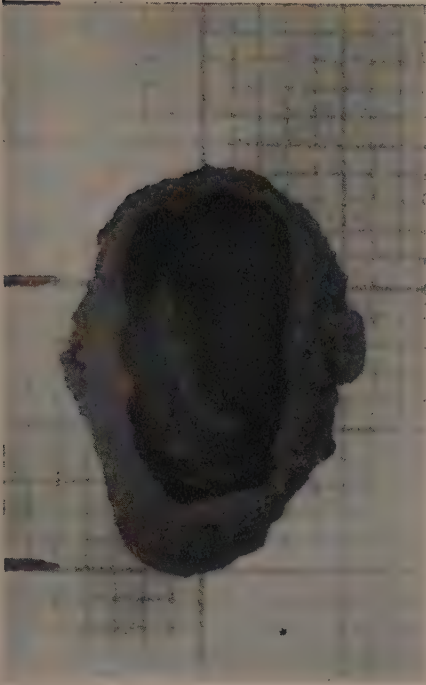


FIG. 1. Pupal cell of *Diparopsis watersi* opened to show the puparium of the parasite, *Carcelia evolans*, and the dead pupa of the host.

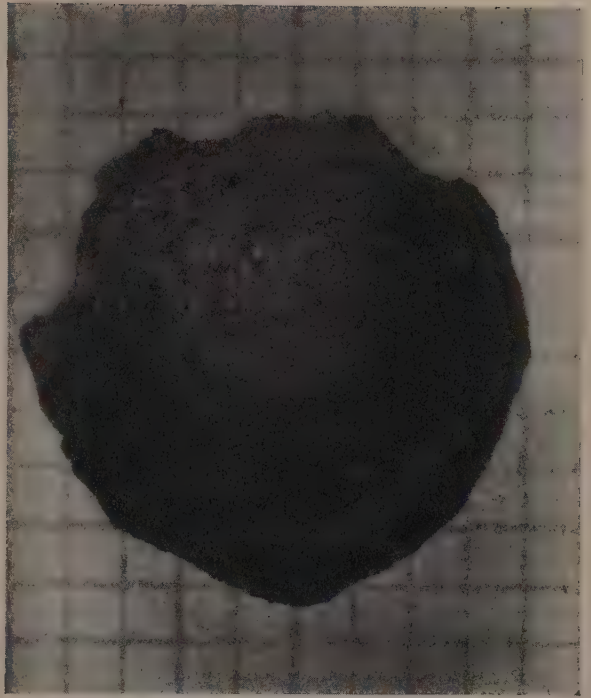


FIG. 2. Interior view of the anterior end of a cell that contained a parasitised pupa of *D. watersi*, showing the exit pit prepared by the fully grown larva of *C. evolans* before it formed its puparium.

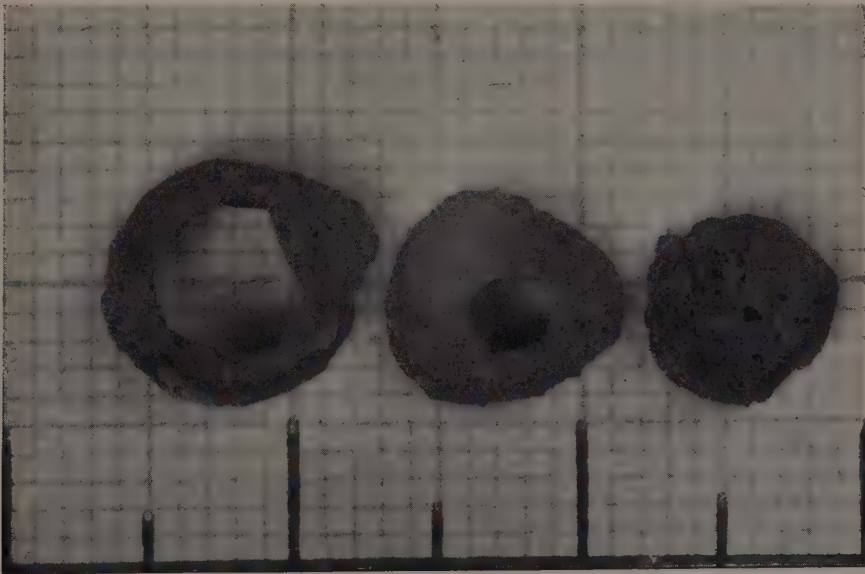


FIG. 3. External views of emergence holes found in pupal cells of *D. watersi*. Left: The large circular hole made by an emerging moth. Centre: The smaller emergence hole of the adult parasite. Right: The minute hole which is often seen at the anterior end of a parasitised pupal cell; in field collections, this hole is superficially similar to holes made in the wall of the cell by predatory ants.

The background to all plates includes a millimetre grid.
 Photographed by A. J. Bennett, Ministry of Agriculture,
 Samaru, Zaria, Northern Nigeria.

TRAPS IN FIELD STUDIES OF *GLOSSINA PALLIDIPIES* AUSTEN.

By J. P. GLASGOW and B. J. DUFFY

*East African Trypanosomiasis Research Organization,
Tororo, Uganda.*

Ford & others (1959) have recently described a version of the fly-round which is an improvement on older methods in that catching procedure is more closely defined, and the results are more amenable to statistical analysis and more easily related to geographical details. Shortcomings in the composition of the fly-round are discussed by Bursell (1961) and an example of its high variability is given by Glasgow (1961a); its chief defect is that it yields so few females that the figures for them are customarily discarded. The study of males alone gives only a partial view of the population dynamics of tsetse flies (*e.g.*, Glasgow & Bursell, 1961) and of their rôle as vectors of pathogens. The fly-round is particularly unsatisfactory in the case of *G. pallidipes* Aust., since of all the species of *Glossina* of economic importance this is probably the least 'available'.* The method of searching for resting flies (Isherwood, 1957; Isherwood & Duffy, 1959a) is a major advance, in that substantial numbers of females are produced. It is, however, expensive, because the yield per man-day is always low; it is very dependent on the skill and zeal of the searcher; and while it can reveal temporal changes in one place it is difficult to use it to compare two different places.

For all these reasons we embarked on a series of experiments to study trap catches of *G. pallidipes* under various conditions and compare them with fly-round catches. The traps used were those designed by Morris & Morris (1949), and the work was done between February 1955 and February 1958 in the Lambwe Valley (0°36' S., 34°19' E.), in the South Nyanza District of Kenya, at an altitude of about 3,900 ft. In the middle of this valley is an evergreen thicket called Ruma, about three square miles in area. Some botanical detail of Ruma is given by Ivens & Cochrane (1956), and Buxton (1955, Plate 24A) gives a photograph.

TABLE I.

Rainfall (mm.) recorded at the Lambwe laboratory, three miles east of and 200 ft. above Ruma.

	1955	1956	1957
January	42.9	120.4	49.3
February	50.8	33.0	70.4
March	60.5	83.5	97.5
April	217.2	284.0	195.7
May	141.2	190.1	244.6
June	45.5	82.3	65.1
July	49.3	73.5	32.1
August	90.7	77.6	106.3
September	197.4	123.2	46.2
October	133.6	112.4	84.5
November	134.6	77.7	122.0
December	97.8	27.0	71.7
Total	1261.4	1284.7	1185.4

* Availability is defined as the proportion of the total male population caught on a standard traverse of a fly-round.

Climatic data are given by Isherwood & others (1961); the mean monthly maximum temperature varies between 27° (June or July) and 31°C. (January or February), and the mean minimum between 15° and 17°C. Rainfall is given in Table I. *G. pallidipes* is very numerous in Ruma. According to Isherwood & others (*in press*) its main source of food is bushbuck, this species alone usually accounting for more than half of the blood-meals identified. Other important hosts are buffalo and bushpig. Several other species of large mammal occur in the grassland round about Ruma and are listed in Table III (footnote).

Fig. 1 is a map of Ruma showing the main vegetation communities. It is a simplified version of an unpublished map prepared from aerial photographs by our colleague, Mr. R. D. Pilson. Three arbitrary paths, known as Nos. 4, 14 and 15, were cut across Ruma from side to side. Nos. 14 and 15 are angled at their N.-W.

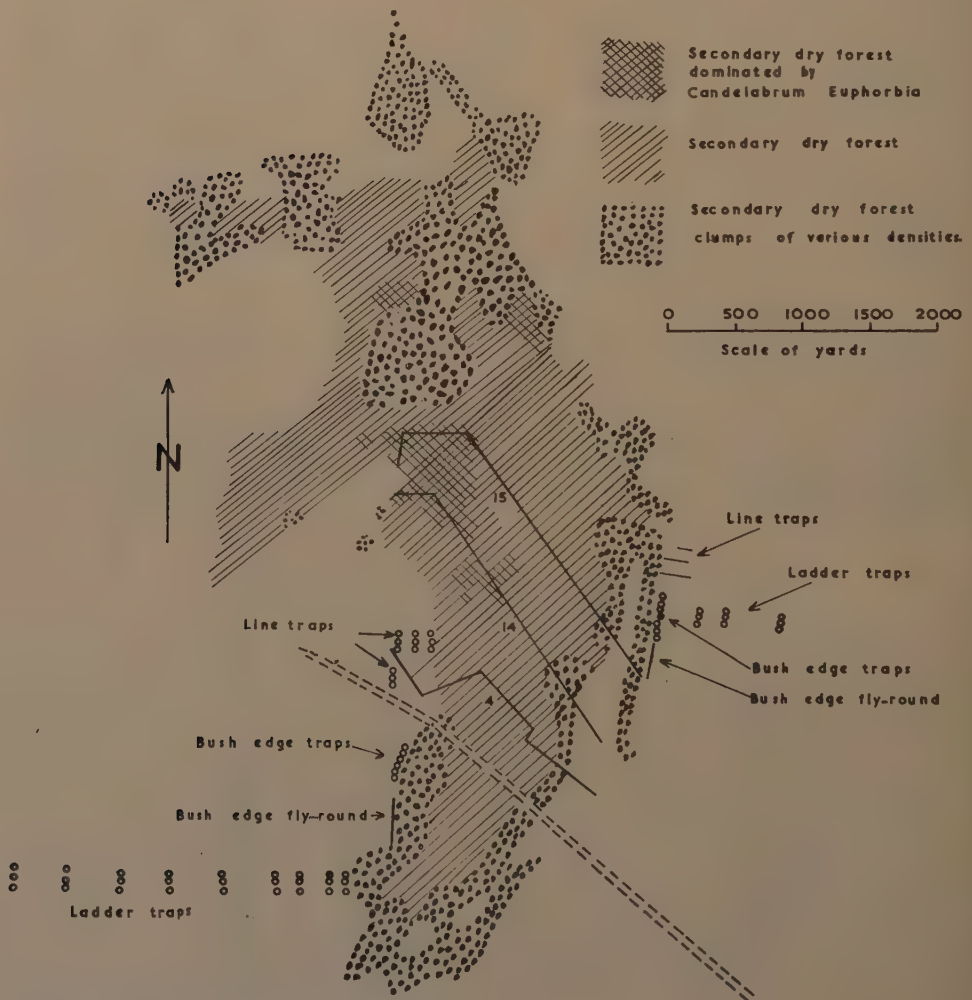


Fig. 1.—Map of Ruma, showing vegetation communities and the location of the various experiments. Unshaded areas represent grassland. For details of arrangement of 'line' traps, see fig. 4.

ends to bring them out into the open. Double corners were introduced into No. 4 in order to discourage flies, from concentrations of *G. pallidipes* at first believed to exist on the edge of Ruma, from penetrating the interior. Subsequent work, however, has shown that there are no such edge concentrations. Paths nos. 4 and 15 were used as fly-rounds, and No. 14 was used for trapping. The location of other traps and fly-rounds is also shown in fig. 1.

Population changes.

Fig. 2 shows, for the period February 1955 to February 1958, the mean apparent density (A.D., defined as the catch of non-teneral males per 10,000 yd. traversed) on fly-rounds 4 and 15 combined; it varied from 70 (August 1956) to 1,300 (January 1958), an 18-fold increase. The broken line shows, for each month, the percentage of the total fly-round catch of non-teneral males that was taken on the catching party, the remainder having been taken from the ground or from vegetation. This 'behaviour criterion' of nutritional status has been shown to have a real meaning, at least in the case of *G. palpalis fuscipes* Newst. (Bursell

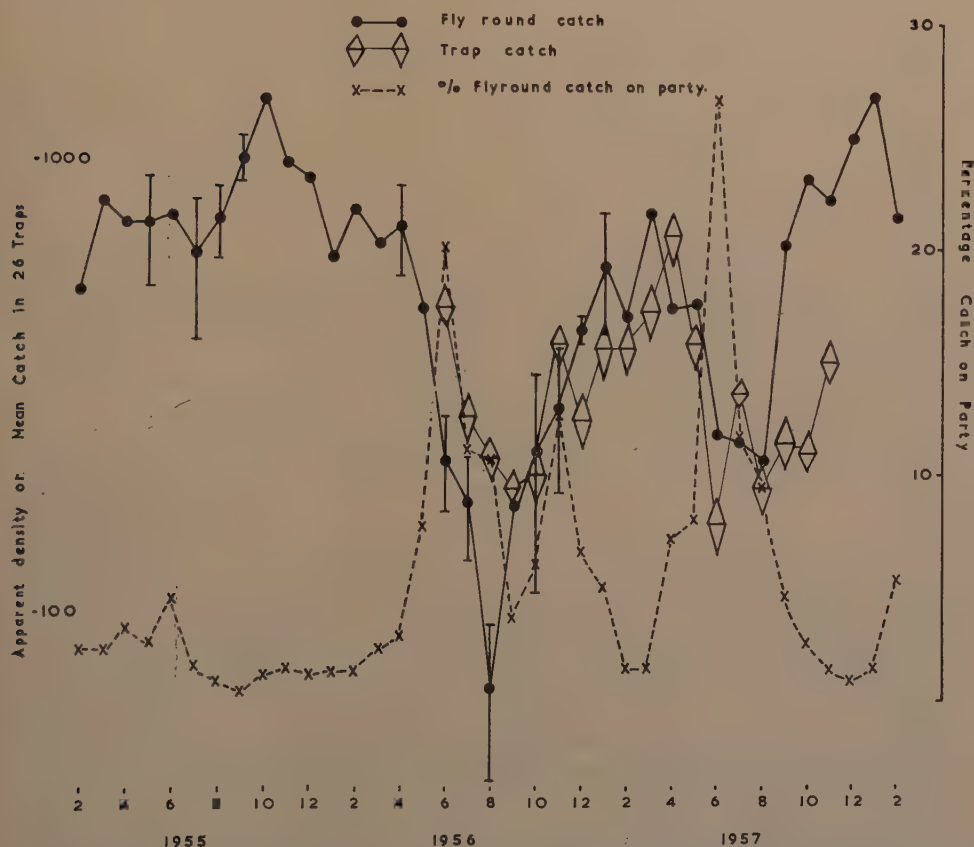


Fig. 2.—Catches of *G. pallidipes* on fly-rounds and in traps, Ruma, 1955–58. Solid circles: mean monthly combined catch on fly-rounds 4 and 15, expressed as apparent density on a logarithmic scale. The ends of each vertical line represent the mean \pm its standard error. Lozenges: mean daily catch in 26 traps on path no. 14, on same logarithmic scale; the horizontal axis is placed at the mean value and the apices of the vertical axis represent the mean \pm its S.E.

& Glasgow, 1960), and to be negatively correlated with apparent density (Isherwood & Duffy, 1959b). This negative correlation was derived from that part of the data considered here which derives from fly-round 15. In certain months the two fly-rounds were done the same number of times (4 or 5), on the same or succeeding days, and in these months they can be treated as one long fly-round. In such months one can calculate the variance of the catch and the standard error of the mean A.D.; in fig. 2, the ends of the vertical lines drawn through the points plotted indicate the monthly mean plus, or minus, its standard error. The magnitude of the standard error is such that doubling (or halving) of the mean catch could be detected, a better result than was obtained with *G. swynnertoni* Aust., in the case of which it was thought that a fly-round 15,000 yd. long would have to be done twice a week to reach this degree of precision (Glasgow, 1961).

In interpreting fig. 2, however, we must consider not only whether the catch differs significantly from month to month, but also whether such differences represent true changes in the population. The low catches in May–December 1956 and June–August 1957 coincide with greatly enhanced values of the percentage of males taken on the catching party, or hunger criterion. It may be that the low catches merely reflect a lower availability of hungry populations, as Isherwood & Duffy (1959b) concluded from the same data, and that there is no convincing

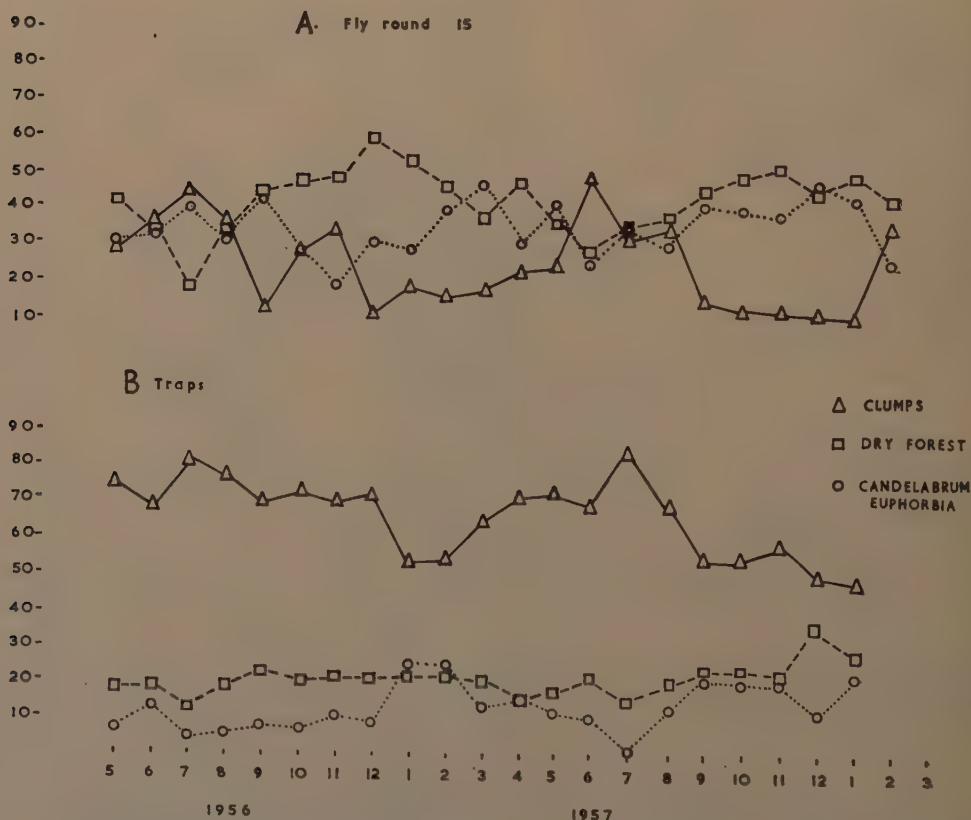


Fig. 3.—The percentage 'popularity' of three vegetation communities, as shown by (A) fly-round 15, (B) traps on path no. 14.

evidence that the population of *G. pallidipes* was other than steady during the period under consideration.

Fig. 2 also shows, for the period June 1956 to November 1957, the mean catches in a total of 26 traps placed along path no. 14. Ten of these traps had been operating since earlier in 1956; sixteen were added in May, in which month the mean catch was 1,063. This value has been discarded because, for reasons not understood, new traps in new sites catch many more flies in the first few weeks than they do subsequently. The mean values plotted are derived from 8–18 observations per month; they vary from 190 (June 1957) to 670 (April 1957). The traps were not cleared every day. Rennison & Smith (1961) found that the total catch was not affected if traps were not cleared every day, provided the traps were protected from ants and other predators. Because our traps were not so protected, and because separate values for each day are required for calculating variances, only those clearings of the traps that were preceded by another clearing 24 hours earlier have been used for calculating the means shown in fig. 2. The standard errors of the trap means tend to be less than those of the fly-round means, but this is a reflection of the fact that they are the means of 8–18 values, as against 4–5 used for the fly-round, and does not indicate that trap catches are intrinsically less variable than fly-round catches.

The trap catches and the fly-round catches show poor agreement: the correlation coefficient of their logarithms (0.40) is not significant. This accords with the conclusions reached by Smith & Rennison (1961). The evidence from the trap catches and the fly-round catches, taken together, does not therefore enable us to conclude definitely that there were real fluctuations in the population.

The distribution of tsetse between the three main vegetation communities appeared different according to the method used to investigate it. Fig. 3 shows the percentage 'popularity' of the three main communities, as revealed by traps and by the fly-round. The secondary dry forest and the candelabrum-*Euphorbia* forest appear about equally popular, judged by either method, but the yields from the clump thickets are very different: by traps, the clumps yielded the most; by fly-rounds, generally the least. Isherwood & Duffy (1959a) studied the distribution of *G. pallidipes* in Ruma from May 1957 to March 1958 by a third technique, that of searching for resting flies. The results obtained by this method agree closely with those obtained by the standard fly-round. Since two methods agree in showing that clumps do not contain particularly large numbers of flies, it must be supposed that the picture presented by the traps is distorted because the clumps happen to contain a high proportion of productive trapping sites.

The influence of site on trap catch.

Previous workers using traps have recognised that small alterations in the position of a trap may have a large influence on the number of tsetse caught (see Buxton, 1955, for earlier work, and Glasgow, 1960). Smith & Rennison (1961), however, found no significant variation between the numbers of flies caught in various sites. We therefore examined the daily catches in 14 traps in one vegetation community, secondary dry forest. These traps were some of those contributing to the data plotted in fig. 3 B. The siting was quite arbitrary; the path was divided into 50-yd. sections by numbered posts, and the traps were placed at either each post or each alternate post. August 1956 was selected for analysis because in this month the largest number of days' observations (18) was made. The catches are given in Table II. Before analysis the catches were transformed to $\log(n+1)$, where n was the number caught. The means given in Table II were calculated from the transformed data. There were large differences between traps; for example, the mean catch of trap 19 was 63 times that of trap 50, and the mean catch of trap 17 was 23 times that of trap 36. The analysis of variance shows that the effect of site is highly significant. It is to be noted that fly-round

14 was a straight line across Ruma, whereas Smith & Rennison's traps were all on a path following the edge of the bush. We believe that this difference accounts for the discrepancy between our results and theirs, both of which were obtained with *G. pallidipes* and Morris traps.

TABLE II.

The daily catches of both sexes of *G. pallidipes* in 14 Morris traps, August 1956.
Means and analysis based on transformed data (see text).

Day of month	Trap number														Daily mean
	17	18	19	20	22	24	26	28	30	32	34	36	50	52	
1	10	21	17	6	3	1	4	1	6	4	3	0	0	6	3.68
2	30	27	21	12	6	7	2	0	11	5	4	0	0	3	4.92
3	12	9	33	6	11	9	6	5	6	6	6	3	0	3	6.14
8	11	2	31	7	7	12	2	1	16	9	4	3	0	1	4.71
9	17	1	15	19	20	18	9	5	18	1	2	0	0	1	4.91
10	12	2	21	4	7	7	4	1	10	1	3	1	0	2	3.50
14	8	1	3	2	2	2	3	0	1	2	3	0	0	2	1.57
15	0	3	3	2	0	0	0	0	0	2	3	0	0	2	0.70
16	10	4	6	0	0	3	1	1	0	0	2	0	0	0	1.02
17	8	19	6	9	5	5	13	1	6	8	3	1	0	4	4.63
21	5	7	7	2	2	4	0	0	1	2	3	1	0	1	1.78
22	30	1	43	5	16	17	10	4	9	6	5	0	0	4	5.98
23	7	3	9	9	0	4	4	0	4	1	0	0	1	0	1.77
24	2	2	0	2	1	4	3	0	5	2	2	0	0	3	1.42
28	8	1	3	10	4	6	1	3	8	7	6	0	0	2	3.06
29	23	0	14	12	1	6	6	4	12	10	2	1	0	5	4.25
30	14	2	50	8	5	13	7	3	8	7	6	1	3	3	6.14
31	13	7	12	20	2	8	4	3	11	11	9	0	1	0	4.78
Trap mean	9.44	3.59	10.69	5.67	3.18	5.40	3.29	1.23	5.29	3.56	3.22	0.41	0.17	1.87	

	Degrees of freedom	Sum of squares	Variance	F	P
Between days	17	9.6099	0.5653	7.80	<0.001
Between sites	13	19.7709	1.5208	20.95	<0.001
Residual	221	16.0201	0.0725		
Total	251	45.4009			

The dispersal of *G. pallidipes* into grassland.

It has been supposed that tsetse see objects in clearings and fly out to them. Du Toit (1954), discussing barrier clearing, stated that "bush should not be visible from one edge of the clearing to the other . . . the object being to achieve a bush-free skyline." On the other hand, Napier Bax (1937) found that *G. swynnertoni* would react to a moving span of oxen at 100 yd. but not at 150 yd. One would suppose on general grounds that the distance at which stationary objects could be perceived would be much less.

The arrangement of traps in lines shown in fig. 4 was set up to test the propositions that *G. pallidipes* perceives traps at a distance and flies out to them, and that it flies more readily along paths cut in long grass. To the east of Ruma there were three lines of traps: line A, 7 traps at 100-ft. intervals in a path 1-2 yd. wide, the most westerly trap being at the junction of the bush and the grassland; line B, 4 traps at 100-ft. intervals, with the trap nearest the bush 100 yd. out along

a straight path 1-2 yd. wide; line C, similar to line B but separated from the bush by undisturbed grass. The numbers entered on fig. 4 are the gross catches in the traps. It is quite clear, from comparison of lines B and C, that the path connecting line B with the bush did not enhance the catch of that line as compared with line C. Comparing lines A and B, the four outer traps of A have catches so similar to line B that one cannot suppose that the three inner traps have

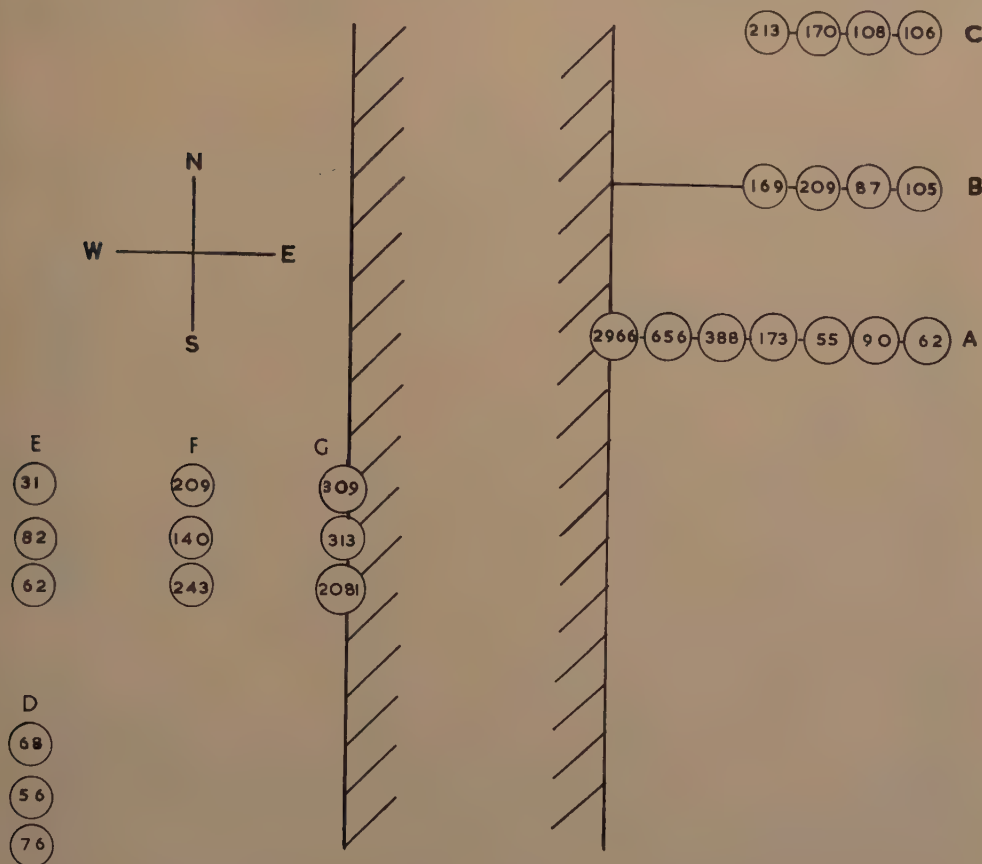


Fig. 4.—Diagram showing arrangement of 'line' traps on either side of Ruma. The position of each trap is indicated by a circle. The numbers in the circles show the total catch of *G. pallidipes* in each trap over 62 weeks, 25.vii.56-3.x.57 (east side) or 50 weeks, 21.x.56-3.x.57 (west side).

intercepted an appreciable number of flies that would otherwise have entered the outer traps. The experiment laid out to the west of Ruma, though differently arranged, led to the same conclusion. Two batteries (D and E) each of three traps, 200 yd. from the bush edge, caught the same number of tsetse, although one battery (E) had two intercepting batteries (F and G), each of three traps, between it and the bush.* It is inferred that traps do not attract flies from a distance, but catch flies that happen anyway to be in their vicinity. The immediate stimulus to enter the traps must, of course, be visual, since the traps

* This arrangement is elsewhere termed a 'ladder' (see p. 802).

are not scented, but evidently the stimulus is effective over a quite short distance, such as five yards, which was the standard distance of the 'bush edge' traps (see p. 805) from the actual edge of the bush.

The arrangement of what were termed 'ladder' traps (see fig. 1) was set up to investigate the depth of dispersal. Each 'rung' of the 'ladder' consisted of three traps, 100 yd. apart, at a given distance from the edge of Ruma bush and a 'ladder' was set up on both the east and the west sides of this thicket. The catches over 66 weeks, with, on the east, preliminary data for 20 weeks over certain distances only, are given in Tables III and IV. To the east (Table III), catches are not

TABLE III.

Dispersal of *G. pallidipes* to the east from Ruma.
Total catch in 4-week period in 3 traps at given distance from edge of bush.

Periods of 4 weeks ending	Distance from bush (yd.)					Animals sighted	
	5	100	300	500	900	Small*	Big*
3.iii.56	—	277	—	15	6	3	43
30.iii.56	—	78	—	3	1	5	123
28.iv.56	—	47	—	4	0	7	52
24.v.56	—	12	—	1	0	6	5
20.vi.56	—	37	—	0	1	0	7
20.vii.56	1064	137	17	2	0	0	16
17.viii.56	1323	61	5	0	0	0	0
14.ix.56	762	12	1	1	0	0	0
12.x.56	438	15	0	0	0	0	0
9.xi.56	360	19	0	1	0	0	6
7.xii.56	341	7	0	0	0	0	0
4.i.57†	1852	49	10	1	0	0	0
1.ii.57	306	1	0	0	0	3	49
1.iii.57	570	7	0	0	1	0	29
29.iii.57	1459	80	15	0	0	47	21
26.iv.57	1105	86	14	0	5	0	17
24.v.57	1410	38	5	0	0	0	0
21.vi.57	523	13	0	0	1	0	6
19.vii.57	250	19	4	2	1	1	51
16.viii.57	68	18	1	2	2	7	12
13.ix.57	91	16	5	9	0	3	33
3.x.57	132	13	3	0	0	8	29
(3 weeks)							
Total (July 1956—Oct. 1957)	12054	591	80	18	10		

* Small animals: bushbuck, bushpig, duiker, impala, oribi, reedbuck. Big animals: hartebeest, roan, topi, waterbuck.

Spoor of buffalo were often seen but the records refer only to sightings made by the party emptying the traps.

† The grassland to the east of Ruma was burnt on the 22nd December 1956.

given at distances greater than 900 yd., because the presence of isolated thickets containing small numbers of *G. pallidipes* increased the catches at the greater distances. To the east of Ruma a barrier clearing had been made in 1950. This had involved the removal of all trees and a number of scattered clump thickets from the grassland. The thickets were regenerating but in 1957 were only about six feet high, a height which does not harbour *G. pallidipes* in Lambwe. To the west, no clearing had been done. There were very few thickets in the vicinity of the western series of 'ladder' traps; the grassland here was lightly wooded, the

most common tree being *Balanites aegyptiaca*. No detailed correspondence was noted between sightings of animals and catches in traps, and inspection of Tables III and IV shows that animals were not especially rare during periods of minimum dispersal. This led us to suppose that the obviously greater dispersal to the west should be ascribed to the vegetational difference, but it is clear from Tables III and IV that animals were more abundant in the west, so that the question must be left open.

TABLE IV.

Dispersal of *G. pallidipes* to the west from Ruma.
Total catch in 4-week period in 3 traps at given distance from edge of bush.

Periods of 4 weeks ending	Distance from bush (yd.)									Animals sighted	
	5	100	300	500	900	1300	1700	2100	2500	Small*	Big*
20.vii.56	1169	36	44	24	30	16	7	12	20	8	6
17.viii.56	1120	21	16	4	7	0	1	0	2	10	27
14.ix.56	817	9	1	4	3	1	0	0	0	1	141
12.x.56	562	38	4	0	0	0	0	0	0	19	47
9.xi.56	742	62	6	5	0	1	0	0	0	5	128
7.xii.56	538	73	3	0	0	0	0	0	0	4	253
4.i.57†	748	4	0	0	0	0	0	0	0	108	350
1.ii.57	472	1	0	1	0	0	0	0	0	320	378
1.iii.57	1525	4	1	0	0	0	0	0	0	638	231
29.iii.57	2537	60	2	1	3	0	0	1	0	83	237
26.iv.57	1228	68	6	6	1	0	3	2	0	76	383
23.v.57	1454	147	12	14	5	3	9	5	1	3	108
21.vi.57	1030	135	33	13	3	6	3	0	4	2	186
19.vii.57	521	70	10	7	1	1	0	0	0	14	52
16.viii.57	418	104	11	9	3	3	1	0	0	28	47
13.ix.57	305	63	1	1	0	1	0	0	1	0	7
3.x.57	231	70	3	0	0	0	0	0	0	0	0
Total	15417	965	153	89	56	32	24	20	28		

* See footnote, Table III.

† The grassland to the west of Ruma was burnt on the 18th December 1956.

Both 'line' traps and 'ladder' traps give information on the variation of dispersal with season. Dispersal is measured by the catches in traps at distances of 100 yd. or more from the edge of the bush. The data are presented in fig. 5. Maximum dispersal was seen to occur in the wet, cool half of the year, when the grass is long. At the time of minimum dispersal the traps were most conspicuous, the grass having been burnt on both sides of Ruma in December 1956. It is to be noted that from August 1956 to February 1957, while the catches in the traps at 100 yd. or more were decreasing, the catches in Ruma on both the fly-rounds and in traps were tending to increase (fig. 2). Thus the very big changes shown in fig. 5 are not a reflection of population changes in Ruma, but represent a change in the degree of dispersal.

Traps are particularly suited for measuring dispersal, because the number of flies involved is very small. Traps catch both sexes, and operate continuously, so that a trap can catch many more flies in a week than can a man. For this reason traps are particularly useful whenever *G. pallidipes* is very rare. Thus, when Thomson, Glover & Trump (1961) nearly exterminated *G. pallidipes* with an insecticide in another part of Lambwe, fly-round catches became zero for a considerable time, but traps, when introduced, quickly demonstrated the existence of a small residual population.

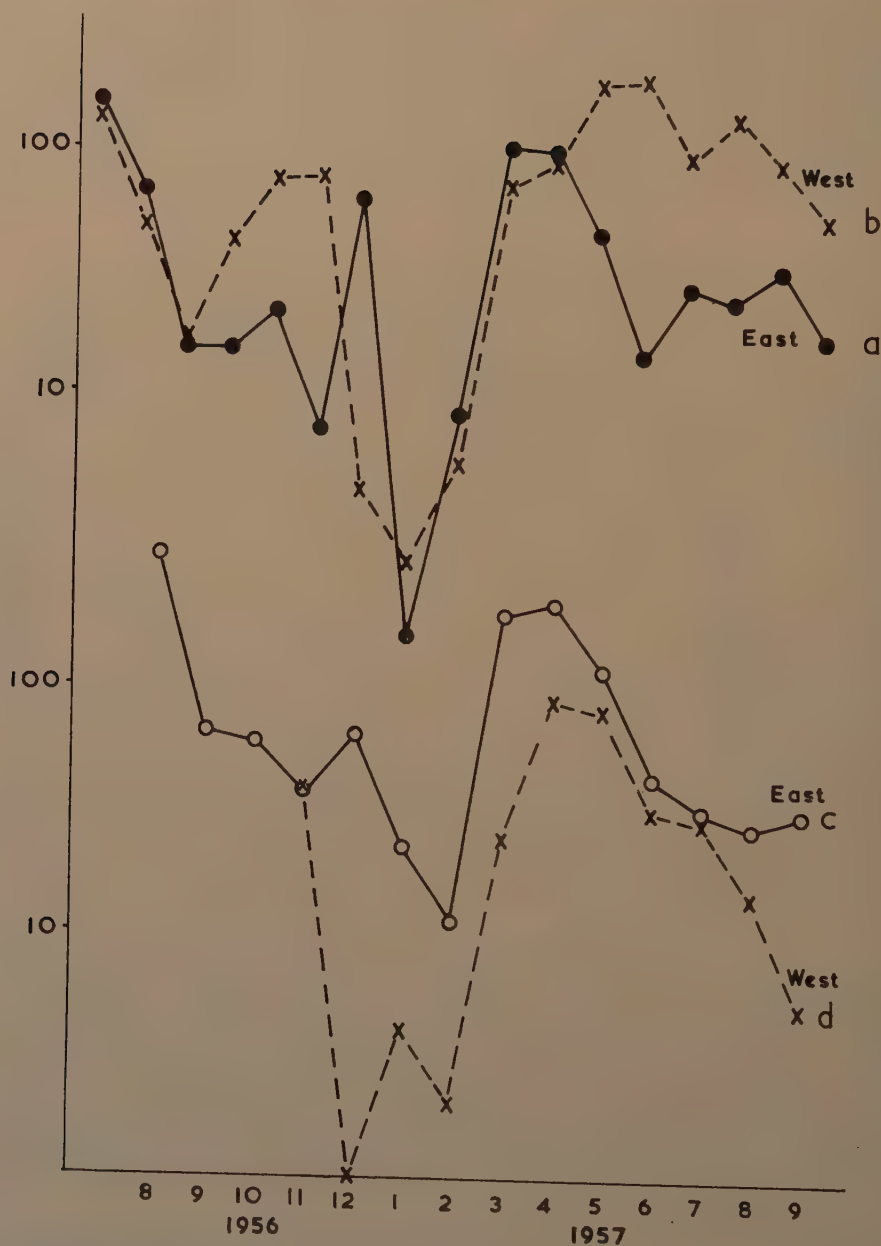


Fig. 5.—Seasonal dispersal of *G. pallidipes*. a,b: dispersal measured by 'ladder' traps; total catch in 4-week periods of all traps 100 yd. or more from bush edge to the east (a) and to the west (b). c,d: dispersal measured by 'line' traps; c, total catch in 12 traps 100-200 yd. from bush edge; d, total catch in 6 traps 200 yd. from bush edge. For arrangement of 'line' traps, see fig. 4. Catches given on logarithmic scale.

The relation between time of day and age of captured flies.

Lewis (1956) found that there was a relation between the age of *Simulium damnosum* Theo. and the time of day at which it is caught. To find out if anything similar occurs with *Glossina*, collections were made on the west edge of Ruma throughout the day from a row of traps and from a short fly-round nearby (see fig. 1). To estimate the relative age of the various samples, the wings were examined and each was assigned to one of the fray categories of Jackson (1946). The mean values (root mean percentage fray) are shown in Table V; there is at

TABLE V.

Wing fray of samples of *G. pallidipes* collected at different times of day, expressed as square root of the mean percentage fray.

Source	Sex	No. of flies	Time				
			1000	1200	1400	1600	1800
Trap ..	♂	135	3.6	2.9	3.3	3.4	3.6
Trap ..	♀	369	2.7	2.5	2.6	3.0	2.3
Fly-round	♂	191	3.1	2.6	2.7	2.7	—

present no way of converting these values to absolute ages, but they are an indication of the relative age of the various samples. In the case of males of *G. morsitans*, under the conditions of Jackson's experiment, a value of 2.3 corresponded to 17 days, of 2.7 to 20 days and of 3.6 to 28 days. There is no perceptible trend in Table V, and it is concluded that there is no tendency in *G. pallidipes* for young individuals to be captured at a particular time of day, either on fly-rounds or in traps. This is not the same thing as saying that the flies feeding at different times of day were all of the same age. This question can be answered only by making a 'biting catch', that is, a catch in which flies are not caught unless they feed. In the work reported here, traps were of considerable help, in that they produced a female sample of reasonable size. However, it is known that a fly-round catch cannot be equated with a 'biting catch' (Bursell, 1961); as to whether this is the case with traps there is no evidence.

Comparison of catches made by traps and by fly-boys.

A line of seven traps, termed 'bush edge' traps, 50 yd. apart, was placed on the eastern side of Ruma and 5 yd. from the bush edge, and to the south of it a short fly-round was laid out, consisting of seven sections each 50 yd. long, in which catching took place only at the numbered posts separating the sections (see fig. 1). The traps were not placed on the fly-round, since the traps and fly-boys might have had interactions that could not be disentangled, and the positions of the traps and the fly-round could not be alternated daily because of administrative difficulties. The fly-round was patrolled six times daily, usually for five days a week, starting at 0730, 0930, 1130, 1330, 1530 and 1730 hr. East African Standard Time. In the longitude of Lambwe the sun rises within 15 minutes of a mean time of 0643 E.A.S.T.; the starting time, 0730, was therefore about three quarters of an hour after sunrise. Each patrol took 35 to 55 minutes, depending on the size of the catch, and immediately after it the traps were cleared. Wet- and dry-bulb temperature readings were taken with a whirling psychrometer at the beginning of the fly-round and again after the traps had been cleared, i.e., 12 times a day. One pair of fly-boys did the first three fly-round patrols and trap clearances, and another pair the remainder. Smith & Rennison

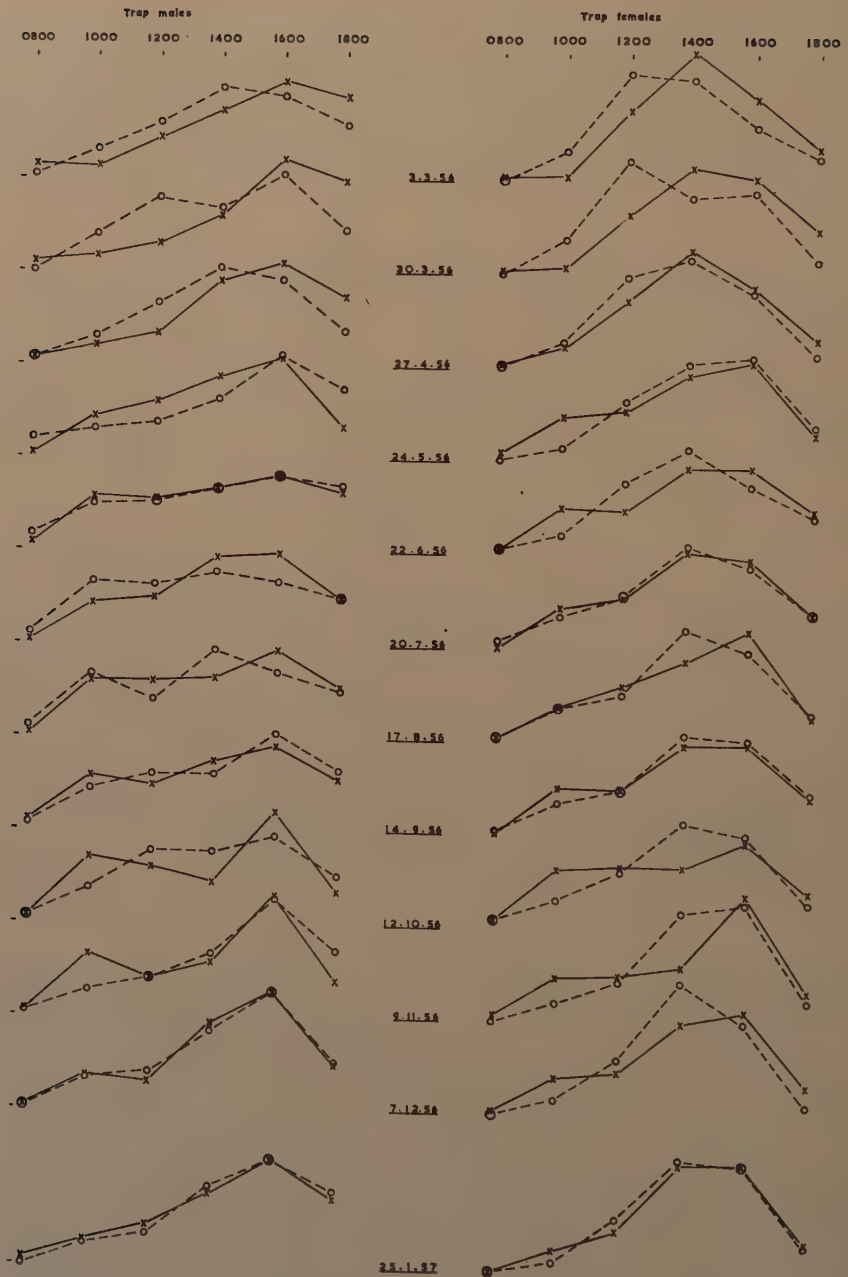


Fig. 6.—The percentage of the total trap catch of males and females of *G. pallidipes* taken at different times of day along the bush edge, Ruma. Continuous line, east side; broken line, west side. Each line refers to the total catch in 4 weeks (usually 20 working days) ending on the date given.

(1961) have recently shown differences to exist even between teams of fly-boys. No provision for the detection of such differences was made in the design of the experiment described here; such differences, however, though they may have obscured effects that might otherwise have become apparent, are unlikely to have produced any systematic bias in the results, since the individuals involved changed from time to time in accordance with a general policy of rotation, with normal wastage and with leave movements. All these arrangements were duplicated on the west side of Ruma (see fig. 1).

The observations continued for 12 months, from February 1956 to January 1957, and the gross catches, arranged in 4-week periods, are given in Table VI.

TABLE VI.

Catches of non-teneral males and females of *G. pallidipes*, in 4-week periods, by traps and by fly-boys operating on the east and west edges of Ruma.

Period ending	Traps				Fly-boys			
	East		West		East		West	
	Males	Females	Males	Females	Males	Females	Males	Females
3.iii.56	889	3221	1055	4121	563	84	2096	92
30.iii.56	550	2019	493	1804	335	73	982	68
27.iv.56	591	3256	330	2332	761	84	608	57
24.v.56	258	1090	459	2186	1091	186	801	131
22.vi.56	410	1711	1062	2649	1609	463	1581	609
20.vii.56	447	1417	610	804	1043	274	1018	462
17.viii.56	236	549	259	442	757	187	560	170
14.ix.56	172	374	154	419	511	54	350	128
12.x.56	103	255	120	376	171	95	374	28
9.xi.56	191	509	217	744	255	99	385	39
7.xii.56	79	335	206	702	324	28	395	24
25.i.57	118	267	273	1081	238	16	406	22
Total	4044	15003	5238	17660	7658	1643	9556	1830

In the traps, 78 per cent. of the flies caught were females; with fly-boys, 17 per cent. were females. The total catch in the traps was 41,945 and by fly-boys 20,687, but as the fly-boys were catching only between one quarter and one half of the time, their rate of catching exceeded that of the traps.

Fig. 6 shows the trap catches at different times of day, for both sexes on both sides of Ruma. Preliminary experiments on the east edge of Ruma had produced peak catches at either 1400 or 1600 hr. This suggested that the peak catch was related to the appearance of the trap as seen from the bush with the sun behind the observer's eye. If this were so, traps on the west edge of Ruma would assume such an appearance during the forenoon. Fig. 6 shows that this expectation was partly fulfilled for the first three 4-week periods, in that the west peak was earlier than the east peak. The west peak, however, was not always before noon and in the fourth and subsequent four-week periods the results were less regular. The values plotted in fig. 6 are the percentage catches at different times of day. Statistical comparisons of the time patterns were made by applying the $2 \times n \chi^2$ test to the original data. The results are summarised in Table VII. In certain periods, such as that ending 9.xi.56, the time pattern was quite different on the two sides of Ruma, with a tendency to two peaks, at 1000 and 1600 hr., on the east side of Ruma, and only one on the west side. In such cases it would be misleading to state that the peak catch is earlier on one side, and the position has been entered in Table VII merely as "different". The double peak is not apparently related to temperature or saturation deficit, of which the mean values,

TABLE VII.

Comparison of the time pattern of trap catches of *G. pallidipes* on two sides of Ruma. The table shows, for each sex, the side on which the catch was the earlier (see fig. 6).

4-week period ending	Males	Females
3.iii.56	West**	West**
30.iii.56	West**	West**
27.iv.56	West**	West**
24.v.56	East**	East**
22.vi.56	Different*	West**
20.vii.56	West**	West**
17.viii.56	Different*	—
14.ix.56	—	—
12.x.56	Different*	Different***
9.xi.56	Different**	Different***
7.xii.56	—	Different***
25.i.57	—	West*

"Different" signifies that the patterns differed on the two sides but not in such a way that either could be described as the earlier.

* $P < 0.05$ ** $P < 0.01$ *** $P < 0.001$

calculated over 28-day periods for each of the hours shown in the figures, invariably had only a single peak, near the middle of the day. It is interesting that there are no proved discrepancies between the sexes, that is to say, no cases where males are significantly earlier on one side and females significantly earlier on the other.

The sexes may be compared in the same way in respect of the time pattern of their entry into the traps. The results are summarised in Table VIII. In 16 out of 24 comparisons, females were caught earlier than males. In five compari-

TABLE VIII.

Comparison of the sexes of *G. pallidipes* in respect of the time pattern of their entry into traps on two sides of Ruma. The table shows, for each side, the sex that appeared the earlier (see fig. 6).

4-week period ending	East	West
3.iii.56	Females***	Females***
30.iii.56	Females***	Females***
27.iv.56	Females***	Females***
24.v.56	—	Females***
22.vi.56	Females***	Females***
20.vii.56	—	Different***
17.viii.56	Different***	Different***
14.ix.56	Females*	Females***
12.x.56	—	Females*
9.xi.56	—	Females**
7.xii.56	—	Females***
25.i.57	Females*	Females***

"Different" signifies that the pattern differed in the two sexes, but not in such a way that either could be described as the earlier.

* $P < 0.05$ ** $P < 0.01$ *** $P < 0.001$

sons the difference was insignificant and in three it was complicated because the males alone had a tendency to two peaks. There is thus a general tendency for females to be caught earlier than males in traps.

Fig. 7 shows the relation between fly-round catch and time of day. As in the case of trap catches, statistical comparisons have been made on the original data, and these are summarised in Table IX. The male peak was earlier in the east

TABLE IX.

Comparison of the time pattern of fly-round catches of *G. pallidipes* on two sides of Ruma. The table shows, for each sex, the side on which the catch was the earlier (see fig. 7).

4-week period ending	Males	Females
3.iii.56 ..	East***	East***
30.iii.56 ..	Different***	East***
27.iv.56 ..	East***	East*
24.v.56 ..	East**	—
22.vi.56 ..	West**	—
20.vii.56 ..	West**	West**
17.viii.56 ..	—	Different*
14.ix.56 ..	West***	—
12.x.56 ..	Different*	—
9.xi.56 ..	East**	—
7.xii.56 ..	Different***	—
25.i.57 ..	—	—

"Different" signifies that the pattern differed on the two sides but not in such a way that either could be described as the earlier.

* $P < 0.05$ ** $P < 0.01$ *** $P < 0.001$

TABLE X.

Comparison of the sexes of *G. pallidipes* in respect of the time pattern of fly-round catches on two sides of Ruma. The table shows, for each side, the sex that appeared the earlier (see fig. 7).

4-week period ending	East	West
3.iii.56 ..	Females***	Different***
30.iii.56 ..	Females***	—
27.iv.56 ..	Different***	Different*
24.v.56 ..	Females***	Females***
22.vi.56 ..	—	Different**
20.vii.56 ..	—	Females*
17.viii.56 ..	—	Females*
14.ix.56 ..	Different*	—
12.x.56 ..	—	—
9.xi.56 ..	—	—
7.xii.56 ..	—	—
25.i.57 ..	—	—

"Different" signifies that the pattern differed in the two sexes, but not in such a way that either could be described as the earlier.

* $P < 0.05$ ** $P < 0.01$ *** $P < 0.001$

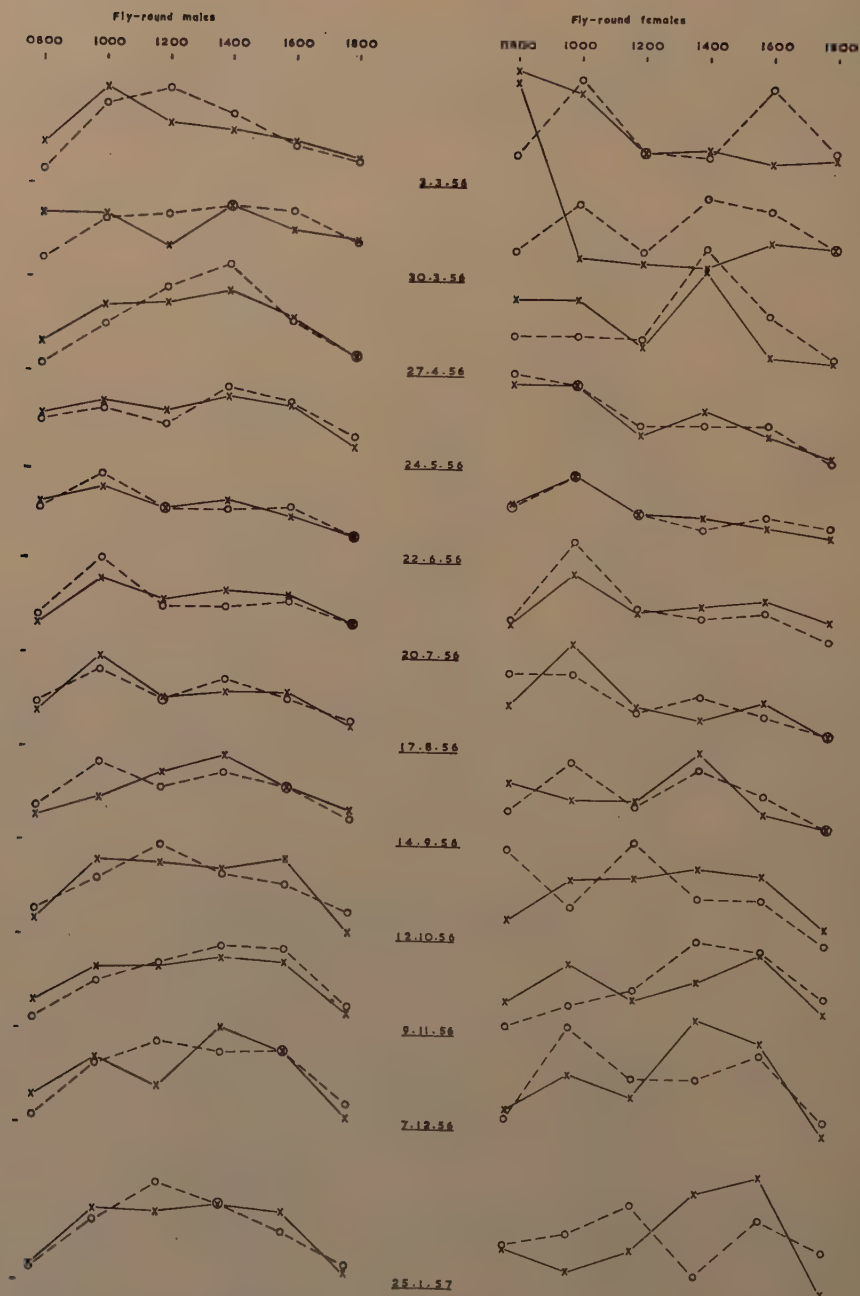


Fig. 7.—The percentage of the total fly-round catch of males and females of *G. pallidipes* taken at different times of day along the bush edge, Ruma. Continuous line, east side; broken line, west side. Each line refers to the total catch in 4 weeks (usually 20 working days) ending on the date given.

than in the west in four periods, and earlier in the west in three periods. The female peak was earlier in the east than in the west in three periods, earlier in the west once. It is interesting that, as with trap catches, there were no discrepancies between the sexes; in no period did the sexes differ significantly as regards the side on which they were earlier.

Table X summarises the comparison between the sexes in respect of the time pattern of fly-round catches on the two sides of Ruma. There were significant differences between the sexes in 11 periods; in six of these the females were caught earlier in the day than males, but in five the patterns were so unlike that they are recorded in Table X merely as "different". Very generally, however, a tendency for females to be caught earlier in the day is discernible in both trap and fly-round catches (Tables VIII and X).

The difference between the traps and the fly-round as regards the pattern of captures is so clear-cut as regards male flies that it need not be tabulated. The fly-round was significantly the earlier in every case (in all but three cases at

TABLE XI.

Method yielding the earlier pattern of captures of females of *G. pallidipes* on two sides of Ruma (see figs. 6 & 7).

4-week period ending	East	West
3.iii.56 ..	Fly-round***	Different***
30.iii.56 ..	Fly-round***	Different***
27.iv.56 ..	Different***	Different***
24.v.56 ..	Fly-round***	Fly-round***
22.vi.56 ..	Fly-round***	Fly-round***
20.vii.56 ..	Fly-round***	Fly-round***
17.viii.56 ..	Fly-round***	Fly-round***
14.ix.56 ..	Fly-round***	Fly-round***
12.x.56 ..	—	Fly-round***
9.xi.56 ..	Fly-round***	—
7.xii.56 ..	—	Fly-round**
25.i.57 ..	—	—

"Different" signifies that the patterns yielded by the two methods differed significantly, but not in such a way that either could be described as the earlier.

* $P < 0.05$ ** $P < 0.01$ *** $P < 0.001$

$P < 0.001$). Similarly, in the case of female flies (Table XI), the pattern of the fly-round captures was earlier than that of the traps during 15 out of the 24 periods recorded, and was never later. Thus the daily pattern of captures of both sexes on fly-rounds had a clear tendency to be earlier than that in traps.

The catching rate of traps and men.

The comparison made in the preceding section and in Table VI between the catches made in seven traps and on 350 yd. of fly-round is not a comparison of the catching rates of traps and men, because the trap catch might well have been doubled by placing traps at 25-yd. instead of 50-yd. intervals. A direct comparison was made in December 1956, on the east edge of Ruma, between two traps and a standing catch by three men who stood all day in one place and caught all the tsetse they could see. Under these conditions movement is not eliminated and is doubtless one of the stimuli attracting tsetse to the spot, but the area sampled is

limited to the vicinity of the catching site. Table XII shows that each trap caught only about half as many males as the three men, but as the men caught almost no females, the total catching rate of each trap was more than twice that of the three men.

TABLE XII.

Comparison of catches of *G. pallidipes* made by two traps and a standing party of three men, on east edge of Ruma, each catching 11 hr. daily.

Period		Traps		Men	
		Males	Females	Males	Females
10-14.xii.56	..	50	137	47	1
17-21.xii.56	..	42	119	30	1
Total	..	92	256	77	2

Discussion.

It is difficult to reach a firm conclusion from the work described here. This is not to disparage traps; figs. 6 and 7 may be confusing, but they must be in some measure a reflection of the vagaries of tsetse fly which could not be glimpsed at all without traps. It was necessary to make reservations even as to whether an eighteen-fold change in fly-round catches indicated a change in the true population. Trap catches changed from 190 to 670; it would be rash to claim that this reflected a population change until more is known about the reasons for which *G. pallidipes* enters traps. Work on *G. pallidipes* in Uganda (Glasgow, 1961b) provides a probable reason for some of the difficulty of obtaining reproducible results: it was found that comparison of catches on successive days showed a confusing pattern of vague and ill-defined patches of flies moving about in an unpredictable fashion. This phenomenon may perhaps contribute to the differences between catches on the east and west sides of Ruma.

Summary.

In order to evaluate Morris traps as a method of investigating populations of *Glossina pallidipes* Aust., and to compare them with fly-rounds, a series of catches was made by both methods in and adjacent to an area of evergreen thicket covering about three sq. miles in the Lambwe Valley, South Nyanza District, Kenya. During the period of the investigations (February 1955–February 1958), the mean monthly apparent density (catches of non-teneral males per 10,000 yd. traversed) determined from a fly-round 5,200 yd. long traversing the thicket varied from 70 to 1,300, and the mean monthly trap catch, determined from 26 traps along a similar traverse but over a shorter period, varied from 190 to 670. Catches by the two methods showed similar fluctuations but were not significantly correlated, and it is not certain that real changes in population occurred. The distribution of *G. pallidipes* between the three main vegetation communities sampled appeared different when studied by traps, or by other methods (fly-rounds, and searching for resting flies). The two latter methods were in agreement, but traps gave different results, perhaps because one of the vegetation communities may have had a high proportion of productive trapping sites.

Dispersal of *G. pallidipes* into the surrounding, sparsely wooded grassland was studied by traps arranged in lines extending east and west of the thicket and at right angles to its edge. Traps appeared not to attract flies over a distance as great

as 100 yd., but to catch only those that chanced to be in their immediate vicinity. Total catches in three traps over 10 months at distances of 5–100–300–500 and 900 yd. west of the thicket edge were 15,417–965–153–89 and 56, respectively; smaller numbers were taken up to 2,500 yd., the greatest distance investigated. Dispersal was greatest in the wet, cool half of the year.

In catches of *G. pallidipes* made in six consecutive two-hour periods daily for one year in two batteries, each of seven traps, and on two short fly-rounds, one of each along the east and the other along the west edge of the thicket, the traps yielded 78 per cent. females and the fly-rounds only 17 per cent. There was a marked tendency for females to be caught earlier in the day than males by either method, and for the pattern of catches to be earlier on fly-rounds than in traps. No difference in age, estimated by wing fray, was found between flies caught, by either method, at different times of day.

The total number of *G. pallidipes* caught by two traps operating 11 hours per day for 10 days at the thicket edge was over four times that caught there concurrently by a stationary party of three men.

It is concluded that traps are valuable because they catch a high proportion of females, thus affording information not given by fly-rounds, and operate continuously, at a higher over-all catching rate than that of men, thus facilitating the study of sparse populations. Nevertheless, site effects and day-to-day variability are both large with traps, so that reproducible results are difficult to obtain.

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vagus, *Anopheles*.
vanderplanki, *Polypedilum*.
vanoyei, *Mansonia*.
variegatus, *Lithobius*.
varius, *Philonthus*.
varius, *Piezotrachelus*.
verbasci, *Anthrenus*; *Bradycellus*.
versicolor, *Mansonia*.
vexans, *Aëdes*.
vicina, *Musca domestica*.
vishnui, *Culex*.
vitripennis, *Nasonia*.
vittatae, *Microctonus*.
vittatus, *Aëdes*.
vomitorea, *Calliphora*.
vuilleti, *Bruchocida*.

W.

watersi, *Diparopsis*.

